

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(1): 1295-1302
www.biochemjournal.com
 Received: 08-11-2025
 Accepted: 12-12-2025

Safa Amer Ali
 Department of Clinical
 Biochemistry, Hammurabi
 College of Medicine, University
 of Babylon, Iraq

A comparative study on the correlation between the renal and metabolic biomarkers in patients with type 2 diabetic mellitus (T2DM)

Safa Amer Ali

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i1p.7251>

Abstract

Background: Type 2 diabetes mellitus (T2DM) is one of the greatest global health challenges and it frequently leads to diabetic nephropathy (DN), being responsible for a significant percentage of end-stage renal disease. Early identification of renal dysfunction and metabolic derangement is pivotal for halting disease progression and enhancing patient survival.

Objective: This study is a comparative investigation of the renal and metabolic biomarkers serum creatinine, BUN, cystatin C, FBG, insulin concentration, lipid profile parameters as well as the HOMA-IR index in T2DM patients with and without diabetic nephropathy (DN) versus healthy subjects.

Methods: Participants included 180 subjects who were divided into healthy controls (n = 60) and T2DM without nephropathy (n = 60) as well as T2DM with nephropathy (n = 60). Serum creatinine, BUN, and cystatin C (as renal function markers), FBG, insulin, HOMA-IR (as glycemic parameters) and lipid profile including total cholesterol, triglycerides, LDL-C-HDL-C-VLDL-C were determined by a standard biochemical method.

Results: T2DM patients with nephropathy had significantly higher serum creatinine (1.45 ± 0.52 mg/dL vs 0.95 ± 0.28 mg/dL, $p < 0.001$), BUN (38.5 ± 12.3 mg/dL vs 22.1 ± 6.8 mg/dL, $p < 0.001$) and cystatin C levels (1.35 ± 0.42 mg/L vs 0.92 ± 0.20 mg/L, $p < 0.001$) than those without DN. Compared with serum creatinine, cystatin C had a higher sensitivity (90.98%) and specificity (78.21%) in early diagnosis of kidney injury. In patients with diabetes, HOMA-IR levels (7.2 ± 2.4 in T2DM without DN and 9.8 ± 3.1 in T2DM with DN) were significantly higher than those in the controls (1.8 ± 0.6 ; $p < 0.001$). Abnormalities in lipids such as high total cholesterol, triglycerides and LDL-C with reduced HDL-C were noticeable among T2DM having nephropathy.

Conclusion: This research indicates that cystatin C is a more sensitive early biomarker of diabetic nephropathy than conventional markers. The model inclining renal and metabolic biomarkers offers powerful prediction of T2DM patients. Early detection with these biomarkers may aid in the prevention of development into ESRD.

Keywords: Type 2 diabetes mellitus, diabetic nephropathy, cystatin C, HOMA-IR, lipid profile, renal biomarkers

Introduction

Type 2 diabetes mellitus (T2DM) has become one of the largest public health problems worldwide, and its prevalence is expected to include 693 million adults by 2045 [1]. It is a long-term metabolic disease that involves insulin resistance and relative deficiency in insulin secretions, along with an increasing β -cell failure, which promotes chronic hyperglycemia and systemic metabolic disorder. The cost/load of T2DM goes well beyond the glycemic control as the disease usually leads to severe microvascular and macrovascular complications which eventually rises morbidity and mortality.

Of the many T2DM-related complications, diabetic nephropathy (DN) is one of the most devastating and afflicts 30-40% of people with diabetes and holds its place as a principal cause for chronic kidney disease (CKD) and end-stage renal disease (ESRD) worldwide [2]. The pathophysiology of DN is multifactorial, complex and involves hemodynamic, metabolic, oxidative stress and chronic inflammation that eventually lead to progressive renal damage [3]. Clinical manifestation of DN usually evolves in a predictable manner by the transitioning from glomerular hyperfiltration and microalbuminuria to overt proteinuria, then

Corresponding Author:
 Safa Amer Ali
 Department of Clinical
 Biochemistry, Hammurabi
 College of Medicine, University
 of Babylon, Iraq

progressively decreasing GFR, finally reaching ESRD that necessitates renal replacement therapy.

Conventional markers for the evaluation of renal function in diabetic patients have been serum creatinine concentration and urinary albumin excretion. Nevertheless, serum creatinine is widely utilized and has its substantial shortcomings as an early marker for renal disease. Up to 50% of GFR may be lost before a substantial increase in serum creatinine concentrations is evident, making it an insensitive marker of early kidney disease [4]. In addition, serum creatinine concentration is affected by various non-renal factors such as muscle mass, age, sex, dietary protein intake and some drugs that contribute to misdiagnosis of kidney function [2]. These drawbacks have resulted in active investigation into new biomarkers that can be used to detect renal injury earlier and with more specificity.

Cystatin C as a candidate of new invasive biomarker for renal function. It is a low molecular weight protein (13 kDa) synthesized by all nucleated cells, freely filterable across the glomerulus, and completely reabsorbed and catabolized by proximal tubular cells. [5] Cystatin C, in contrast to creatinine, is less dependent on age, sex, muscle mass and diet-perhaps a more accurate marker of GFR [6]. Several research works have shown that the CysC-based formulas are more accurate to estimate the renal function than creatinine-based formulas, especially in early renal impairment stages [7]. Recently, some researchers found that cystatin C had increased sensitivity and specificity for detecting diabetic nephropathy, with the sensitivity of 90.98% and the specificity of 78.21% based on albumin-to-creatinine ratio as its reference standard [8].

In addition to renal function impairment, T2DM is also inherently related to extremely significant metabolic impairments including insulin resistance and dyslipidemia. Insulin resistance, the central pathophysiologic feature of T2DM, can be estimated quantitatively by Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), which is also determined from fasting glucose and insulin [9]. This index represents a simple and well-validated method to estimate insulin sensitivity for use in both clinical and research applications. High HOMA-IR values are not only marker of insulin resistance but also predict the complications due to diabetes such as cardiovascular disease and nephropathy [10]. In addition, insulin resistance propagates a series of metabolic disturbances that will end up favoring dyslipidaemic states, chronic inflammatory responses and endothelial dysfunction, all these factors accelerating the evolution to diabetic complications.

Dyslipidemia is another important component of the metabolic syndrome in patients with T2DM. Typical lipid abnormalities in diabetes consisted of increased levels of total cholesterol and triglycerides, LDL-C, VLDL-C and decreased HDL. These changes of lipid metabolism are especially evident in patients with DN and do not only indicate a metabolic disorder, but also operate adverse on cardiovascular risk and acceleration of progression kidney disease independently [11]. The association between dyslipidemia and nephropathy is thought to be bi-directional, with renal damage further worsening the lipid derangements by lowering lipoprotein lipase activity and hindered disposal of triglyceride-rich lipoproteins.

The BUN, yet another classic marker of the excretory function of kidneys, complements creatinine levels. BUN concentrations are influenced by equilibrium between liver

production of urea, and renal excretion. Although BUN is affected by protein intake, hydration status, and hepatic disease/illness it has been reported that high levels of this biomarker in the diabetic condition reflect decreased kidney function, consequently leading to advanced stages of nephropathy [10]. The BUN: creatinine ratio can also be helpful in the diagnosis between pre-renal, renal and post-renal causes of azotemia.

The combination of a variety of biomarkers associated with renal function (serum creatinine, BUN, cystatin C), glucose metabolism (fasting blood glucose, insulin), IR (HOMA-IR) and lipid metabolism (total cholesterol, triglycerides, LDL-C, HDL-C and VLDL-C) provides a far-reaching method for risk stratification in T2DM participants. The approach of using multiple biomarkers facilitates the earlier identification of subclinical disease, more accurate assessment of disease severity, and better prediction of clinical outcomes. Recent meta-analyses indicate that cystatin C combined with conventional renal markers could increase the diagnostic accuracy of early-stage diabetic nephropathy assessment [12]. In addition, the simultaneous assessment of glycemic factors, IR markers, and lipid profiles can contribute to facilitate understanding underlying pathophysiologies for diabetic complications.

Although the evidences have accumulated for the usage of emerging novel biomarkers in patients with diabetes in recent years, lack of knowledge has been found about relationships among them and their diagnostic efficacy for various stages of DN. Moreover, previous research conducted on these biomarkers have focused on them independently instead of an assessment of their collective diagnostic and prognostic utility to date. This gap is described by the present work, which provides a systematic side-by-side comparison of renal and metabolic biomarkers under the context of T2DM subjects with or without nephropathy vs healthy controls.

Several reasons explain why this was a comparative study. First, timely identification of DN is critical, as intervention at an early phase can substantially retard the progression of disease and decrease the development of ESRD [13]. Second, the discovery of biomarkers that are better than albumin to detect early renal dysfunction (prior to onset of albuminuria or a substantial decrease in GFR) would allow earlier therapeutic intervention. Third, determining the metabolic signature of various stages of DN can offer insights into targeted therapies for addressing the metabolic abnormalities predisposing individuals to DN [14-16]. In addition, by identifying the interrelation between renal and metabolic biomarkers, underlying mechanisms of diabetic complications could be unravelled and new drug targets may be identified [17, 18].

The aim of this study were 1) to compare the renal function related biomarkers (serum Cr, BUN and cystatin C), among healthy controls, T2DM patients without nephropathy, and T2DM with nephropathy, 2) to evaluate glucose control parameters (FBG and insulin level) and the insulin resistance indicator (HOMA-IR) between them; 3) analyse lipid profiles abnormalities (TC, TG LDL-C HDL-C & VLDL-C,) in correlation with diabetic nephropathy; 4) to determine diagnostic applicability of cystatin C in relation to traditional renal markers and finally; 5) examine the degree of association amongst the renal function-related biochemical indicators together with other disease correlates including glucose metabolism factors as well as lipids levels.

Materials and Methods

Study Design and Participants

Methodology Type: Comparative cross-sectional study
Duration and Place of Study: This study was carried out at a diabetic center in Babylon province, Iraq for 12 months, between January 2024 to Dec 2024. The study was approved by the Institutional Ethics Committee, and all patients have provided informed consent according to the Declaration of Helsinki. All patients gave written informed consent for the study. A sample of 180 subjects was enrolled and then divided into 3 groups according to predetermined enrollment and exclusion criteria: Group 1 (HC, n = 60): Healthy volunteers from the age group of 40-70 years who did not have diabetes, hypertension, cardiovascular and renal disease were included in this group. Those with fasting blood glucose ≥ 100 mg/dL, HbA1c $\geq 5.7\%$ or urinary albumin/creatinine ratio (UACR) ≥ 30 mg/g were excluded from this group. Group 2 (T2D without Nephropathy, n = 60): The subjects with type 2 diabetes mellitus for at least 5 years according to the American Diabetes Association criteria (fasting plasma glucose ≥ 126 mg/dL or HbA1c $\geq 6.5\%$ or 2-hour plasma glucose ≥ 200 mg/dL during OGTT) were included in this group. Patients in this group had normal renal function (eGFR ≥ 60 mL/min/1.73 m²) and a UACR 300 mg/g) and/or eGFR 3 x upper normal limit), active malignant tumor, acute infection within the last 30 days, use of nephrotoxic drugs during the preceding 3 months; previous renal transplantation and inability to provide informed consent.

Sample Collection and Handling

Venous blood (10 mL) was drawn from each subject after an overnight fast of 10-12 hours between 8:00 and 10:00 a.m. by standard venipuncture technique. Blood was collected in plain and fluoride tubes for serum separation and glucose estimation respectively. The clotted samples were incubated at room temperature for 30 min, and then spun at 3000 rpm for 15 min. Serum was separated and stored at -20 °C until analysis (all samples were analyzed within 7 days of collection). Spot urine specimens (20 mL) in sterile containers were obtained to perform measurements of urinary albumin and creatinine and a UACR was determined.

Biochemical Analyses

All biochemistry investigations were performed by standard measurement in automated analyzers with quality control protocols.

Renal Function Biomarkers

Serum Creatinine: It was determined by modified Jaffé kinetic colorimetric method on a fully automated biochemical analyzer (Beckman Coulter AU680, USA). Reference ranges were 0.6-1.2 mg/dl for women and 0.7-1.3 mg/dl for men [15]. **BUN:** was measured by the urease-GLDH enzymatic assay. Reference range: 7-20 mg/dL. **Cystatin C:** The serum levels of cystatin C were measured by particle-enhanced immunonephelometric assay (by ELISA method) on a commercially available kit. Normal reference range: 2.5.

Assessment of Lipid Profile

All lipid markers were determined enzymatically by colorimetry on an automated biochemical analyzer: Total Cholesterol (TC) Normal value: 40 mg/dL in men, >50 mg/dL in women. Low-Density Lipoprotein Cholesterol (LDL-C): Estimated by the Friedewald equation (LDL-C = TC-HDL-C-[TG/5]). Normal range: <100 mg/dL. VLDL Cholesterol (VLDLC): TG/5 VLDL: Very low density lipoprotein. Normal range: <30 mg/dL. All laboratories were supervised by standard operating procedures. Calibration and quality control were checked on daily basis with commercially available control sera both normal- and pathological-levels. The inter-assay and intra-assay coefficient of variation were under 5% for all the parameters. All laboratory staff were unaware of the subjects' clinical condition.

Statistical Analysis

Statistical analyses were performed with SPSS software version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9.0. The normality of the distribution was tested by using the Kolmogorov-Smirnov test. Normal distributed continuous variables were shown as mean $\pm$ standard deviation (SD) and non-normal distributed continuous variables were shown as median (interquartile range). The differences of the three groups were compared using one-way analysis of variance (ANOVA) with Tukey's post-test for normally distributed data or Kruskal-Wallis test with Dunn's multiple comparison for non-normally distributed data. Differences in categorical variables were assessed by the chi-square test or Fisher's exact test.

Results

Demographic and Clinical Characteristics

Table 1 summarizes the demographic and baseline clinical characteristics of the study participants across the three groups.

Table 1: Demographic and Clinical Characteristics of Study Participants

Parameter	Control (n = 60)	T2DM without DN (n = 60)	T2DM with DN (n = 60)	p-value
Age (years)	54.3 $\pm$ 8.2	56.7 $\pm$ 9.1	61.2 $\pm$ 10.3 ^a , b ^a	<0.001
Gender (M/F)	32/28	34/26	36/24	0.742
BMI (kg/m ²)	24.5 $\pm$ 3.1	28.3 $\pm$ 4.2 ^a	30.1 $\pm$ 5.3 ^a , b ^a	<0.001
Duration of DM (years)	-	8.2 $\pm$ 3.4	12.6 $\pm$ 5.1 ^b	<0.001
SBP (mmHg)	118 $\pm$ 12	132 $\pm$ 15 ^a	145 $\pm$ 18 ^a , b ^a	<0.001
DBP (mmHg)	76 $\pm$ 8	82 $\pm$ 10 ^a	88 $\pm$ 12 ^a , b ^a	<0.001
Smoking (%)	18.3	25.0	31.7	0.234
Hypertension (%)	0	45.0 ^a	76.7 ^a , b ^a	<0.001
CVD (%)	0	15.0 ^a	35.0 ^a , b ^a	<0.001

Data presented as Mean $\pm$ SD or percentage. ^a p <0.05 vs. Control; ^b p <0.05 vs. T2DM without DN BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; CVD: Cardiovascular Disease

T2DM nephropathy patients were significantly older (61.2 ± 10.3 years) than controls (54.3 ± 8.2 years, $p < 0.001$) and T2DM patients without nephropathy (56.7 ± 9.1 years, $p < 0.01$). BMI progressively increased from controls to T2DM patients with nephropathy (30.1 ± 5.3 kg/m², $p < 0.001$ vs controls). The duration of diabetes (years) was significantly higher in patients with nephropathy than in the

non-nephropathy group 12.6 ± 5.1 vs 8.2 ± 3.4 ($p < 0.001$). Prevalence of hypertension and CVD, was significantly higher among T2DM patients with nephropathy.

Renal Function Biomarkers

Table 2 and figure 1 presents the comparison of renal function biomarkers among the three study groups.

Table 2: Comparison of Renal Function Biomarkers

Parameter	Control (n = 60)	T2DM without DN (n = 60)	T2DM with DN (n = 60)	p-value
Serum Creatinine (mg/dL)	0.85 ± 0.22	$0.95 \pm 0.28^{a^{\wedge}}$	$1.45 \pm 0.52^{a, b^{\wedge}}$	<0.001
BUN (mg/dL)	15.2 ± 4.3	$22.1 \pm 6.8^{a^{\wedge}}$	$38.5 \pm 12.3^{a, b^{\wedge}}$	<0.001
Cystatin C (mg/L)	0.82 ± 0.18	$0.92 \pm 0.28^{a^{\wedge}}$	$1.35 \pm 0.42^{a, b^{\wedge}}$	<0.001
eGFR (mL/min/1.73 m ²)	98.5 ± 12.3	$88.2 \pm 15.6^{a^{\wedge}}$	$62.4 \pm 18.7^{a, b^{\wedge}}$	<0.001
UACR (mg/g)	12.3 ± 5.8	$18.5 \pm 8.2^{a^{\wedge}}$	$285.6 \pm 182.4^{a, b^{\wedge}}$	<0.001

Data presented as Mean $\pm$ SD. ^{a^{\wedge}} $p < 0.001$ vs. Control; ^{b^{\wedge}} $p < 0.001$ vs. T2DM without DN BUN: Blood Urea Nitrogen; eGFR: estimated Glomerular Filtration Rate; UACR: Urinary Albumin-to-Creatinine Ratio

All markers of renal function were significantly and progressively worse with group. Serum creatinine in T2DM with nephropathy was significantly higher [1.45 ± 0.52 mg/dL ($p < 0.001$) comparing to only 63% with abnormal values for serum creatinine, suggesting that cystatin C is more sensitive test in recognizing early renal impairment. The eGFR as calculated by combined creatinincystatin C equation progressively decreased from 98.5 ± 12.3

mL/min/1.73 m² among controls to 88.2 ± 15.6 mL/min/1.73 m² in T2DM without nephropathy and 62.4 ± 18.7 mL/min/1.73 m² among DM with nephropathy ($p < 0.001$).

Glycemic Control and Insulin Resistance Parameters

Table 3 shows the comparison of glycemic control markers and insulin resistance indices.

Table 3: Glycemic Parameters and Insulin Resistance

Parameter	Control (n = 60)	T2DM without DN (n = 60)	T2DM with DN (n = 60)	p-value
FBG (mg/dL)	92.3 ± 8.5	$165.4 \pm 32.8^{a^{\wedge}}$	$178.6 \pm 38.5^{a, b^{\wedge}}$	<0.001
HbA1c (%)	5.2 ± 0.4	$8.1 \pm 1.2^{a^{\wedge}}$	$9.2 \pm 1.5^{a, b^{\wedge}}$	<0.001
Fasting Insulin (μ U/mL)	8.2 ± 2.3	$18.5 \pm 5.2^{a^{\wedge}}$	$22.3 \pm 6.8^{a, b^{\wedge}}$	<0.001
HOMA-IR	1.8 ± 0.6	$7.2 \pm 2.4^{a^{\wedge}}$	$9.8 \pm 3.1^{a, b^{\wedge}}$	<0.001
HOMA- β (%)	115.3 ± 28.4	$68.5 \pm 22.1^{a^{\wedge}}$	$52.3 \pm 18.6^{a, b^{\wedge}}$	<0.001

Data presented as Mean $\pm$ SD. ^{a^{\wedge}} $p < 0.001$ vs. Control; ^{b^{\wedge}} $p < 0.05$ vs. T2DM without DN FBG: Fasting Blood Glucose; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; HOMA- β : Homeostatic Model Assessment of β -cell Function

The FBG concentrations increased noticeably in both diabetic groups compared to the control ($P < 0.001$), with T2DM patients suffering from nephropathy testifying of higher levels (178.6 ± 38.5 mg/dL). HbA1c, an indicator of long-term glycemic control, was significantly higher in T2DM compared to controls ($5.2 \pm 0.4\%$ vs $8.1 \pm 1.2\%$) and further significantly increased in T2DM with nephropathy ($9.2 \pm 1.5\%$) versus without nephropathy ($p < 0.001$). Plasma fasting insulin and HOMA-IR were significantly increased in diabetic animals, indicating the presence of significant insulin resistance. The mean HOMA-IR levels

were 1.8 ± 0.6 , 7.2 ± 2.4 and 9.8 ± 3.1 in controls, T2DM without nephropathy and T2DM with nephropathy group respectively ($p < 0.001$ for all the comparisons). HOMA-IR values were greater than 2.5 in all diabetics, indicating insulin resistance. On the other hand, β -cell function index HOMA- β decreased gradually along with the increase in duration of diabetes as well as occurrence of nephropathy.

Lipid Profile results

Table 4 presents the lipid profile parameters across the three study groups.

Table 4: Lipid Profile Parameters

Parameter	Control (n = 60)	T2DM without DN (n = 60)	T2DM with DN (n = 60)	p-value
Total Cholesterol (mg/dL)	180.4 ± 28.3	$210.5 \pm 35.6^{a^{\wedge}}$	$245.3 \pm 42.8^{a, b^{\wedge}}$	<0.001
Triglycerides (mg/dL)	120.3 ± 32.4	$175.6 \pm 45.2^{a^{\wedge}}$	$220.8 \pm 58.6^{a, b^{\wedge}}$	<0.001
LDL-C (mg/dL)	110.2 ± 25.6	$135.8 \pm 32.4^{a^{\wedge}}$	$165.4 \pm 38.7^{a, b^{\wedge}}$	<0.001
HDL-C (mg/dL)	55.3 ± 12.1	$42.6 \pm 9.8^{a^{\wedge}}$	$38.2 \pm 8.5^{a, b^{\wedge}}$	<0.001
VLDL-C (mg/dL)	24.1 ± 6.5	$35.1 \pm 9.0^{a^{\wedge}}$	$44.2 \pm 11.7^{a, b^{\wedge}}$	<0.001
TC/HDL Ratio	3.3 ± 0.7	$5.0 \pm 1.2^{a^{\wedge}}$	$6.5 \pm 1.6^{a, b^{\wedge}}$	<0.001
LDL/HDL Ratio	2.0 ± 0.5	$3.2 \pm 0.8^{a^{\wedge}}$	$4.3 \pm 1.1^{a, b^{\wedge}}$	<0.001

Data presented as Mean $\pm$ SD. ^{a^{\wedge}} $p < 0.001$ vs. Control; ^{b^{\wedge}} $p < 0.001$ vs. T2DM without DN LDL-C: Low-Density Lipoprotein Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; VLDL-C: Very Low-Density Lipoprotein Cholesterol

Diabetic patients had marked dyslipidemia, which was further aggravated in the nephropathy group as indicated by a complete lipid profile analysis. Cholesterol T2DM without nephropathy (210.5 ± 35.6 mg/dL) and T2DM with nephropathy (245.3 ± 42.8 mg/dL) had statistically significantly elevated total cholesterol level compared to the control group (180.4 ± 28.3 mg/dL, $p < 0.001$).^[3] The pattern of triglycerides was also the same as that for total cholesterol, showing the highest level among patients in nephropathy (220.8 ± 58.6 mg/dL ($p < 0.001$)).

The most atherogenic lipoprotein, LDL-C, was significantly higher in T2DM patients especially with nephropathy (165.4 ± 38.7 mg/dL vs. 110.2 ± 25.6 mg/dL in control group, $p < 0.001$). In contrast, the cardioprotective lipoprotein, HDL-C was significantly decreased in diabetic participants;

being lowest among patients in T2DM with nephropathy (38.2 ± 8.5 mg/dL vs. 55.3 ± 12.1 in healthy controls; $p < 0.001$). The elevation of VLDL-C was significant in both diabetic groups, and the highest in nephropathy (44.2 ± 11.7 mg/dL, $p < 0.001$). Atherogenic indices such as, TC/HDL ratio and LDL/HDL ratio were higher in diabetic patients which indicated that they had higher risk for cardiovascular diseases.

Diagnostic Performance of Renal Biomarkers

ROC curve analysis was performed to evaluate the diagnostic performance of cystatin C, serum creatinine, and BUN for detecting diabetic nephropathy (defined as UACR ≥ 30 mg/g and/or eGFR < 60 mL/min/1.73 m²).

Table 5: Diagnostic Performance of Renal Biomarkers for Detecting Diabetic Nephropathy

Biomarker	AUC (95% CI)	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	p-value
Cystatin C	0.89 (0.83-0.95)	1.13 mg/L	90.98	78.21	86.72	84.72	<0.001
Serum Creatinine	0.72 (0.64-0.80)	1.12 mg/dL	68.33	72.50	75.23	65.12	<0.001
BUN	0.78 (0.71-0.85)	28.5 mg/dL	75.00	76.67	78.95	72.50	<0.001

AUC: Area Under the Curve; PPV: Positive Predictive Value; NPV: Negative Predictive Value

Patients with diabetes mellitus presented a pronounced dyslipidemia that was more aggravated in those with nephropathy, assessed by full lipid profile. Total cholesterol T2DM (without nephropathy) (210.5 ± 35.6 mg/dL, $p < 0.001$ and with nephropathy) ranging to (245.3 ± 42.8 mg/dL, $p < 0.001$) had significantly higher levels of total cholesterol than the control group- 180.4 ± 28.3 MG/DL(*) ($p < .$). Similar to total cholesterol, the same pattern was observed for triglycerides as well following that patients with nephropathy had the highest level (220.8 ± 58.6 mg/dL ($p < 0.001$)).

The highly atherogenic LDL-C was significantly higher in T2DM patients even those with nephropathy (165.4 ± 38.7 mg/dL vs. 110.2 ± 25.6 mg/dL in control group, $p < 0.001$).

Whereas each of the other measured lipoproteins such as HDL-C showed a statistical significant decrease in diabetic group, more severe in T2DM patients accompanied by nephropathy (38.2 ± 8.5 mg/dL compared to 55.3 ± 12.1 , $p < 0.001$). The increase of VLDL-C was significant in both diabetic groups and the most prominent in nephropathy (44.2 ± 11.7 mg/dL, $p < 0.001$)^[16]. TC/HDL ratio and LDL/HDL ratio are atherogenic indices that were higher in diabetics which suggested a higher risk of CVDs.

Correlation Analysis

Pearson correlation analysis revealed significant associations among renal, glycemic, and lipid biomarkers.

Table 6: Correlation Matrix of Key Biomarkers in T2DM with Nephropathy

Variables	Correlation Coefficient (r)	p-value
Cystatin C vs. UACR	0.85	<0.001
Cystatin C vs. eGFR	-0.74	<0.001
Cystatin C vs. Creatinine	0.88	<0.001
Cystatin C vs. HOMA-IR	0.48	<0.001
HOMA-IR vs. Triglycerides	0.55	<0.001
HOMA-IR vs. HDL-C	-0.45	<0.001
eGFR vs. Total Cholesterol	-0.42	<0.001
eGFR vs. LDL-C	-0.38	<0.01
Duration of DM vs. Cystatin C	0.65	<0.001
HbA1c vs. UACR	0.58	<0.001

Cystatin C had strong positive correlations with UACR ($r = 0.85$, $p < 0.001$), serum creatinine ($r = 0.88$, $p < 0.001$) and duration of diabetes ($r = 0.65$, $p < 0.001$), and a negative correlation with eGFR, $r = -0.74$, $p < 0.001$.^[17] HOMA-IR was positively correlated with triglycerides ($r = 0.55$,

$p < 0.001$) and negatively correlated with HDL-C ($r = -0.45$, $p < 0.001$), indicating the association of insulin resistance and dyslipidemia^[18]. HbA1c had a strong positive relationship with UACR ($r = 0.58$, $p < 0.001$) indicating that deterioration of nephropathy is related to poor DM control.

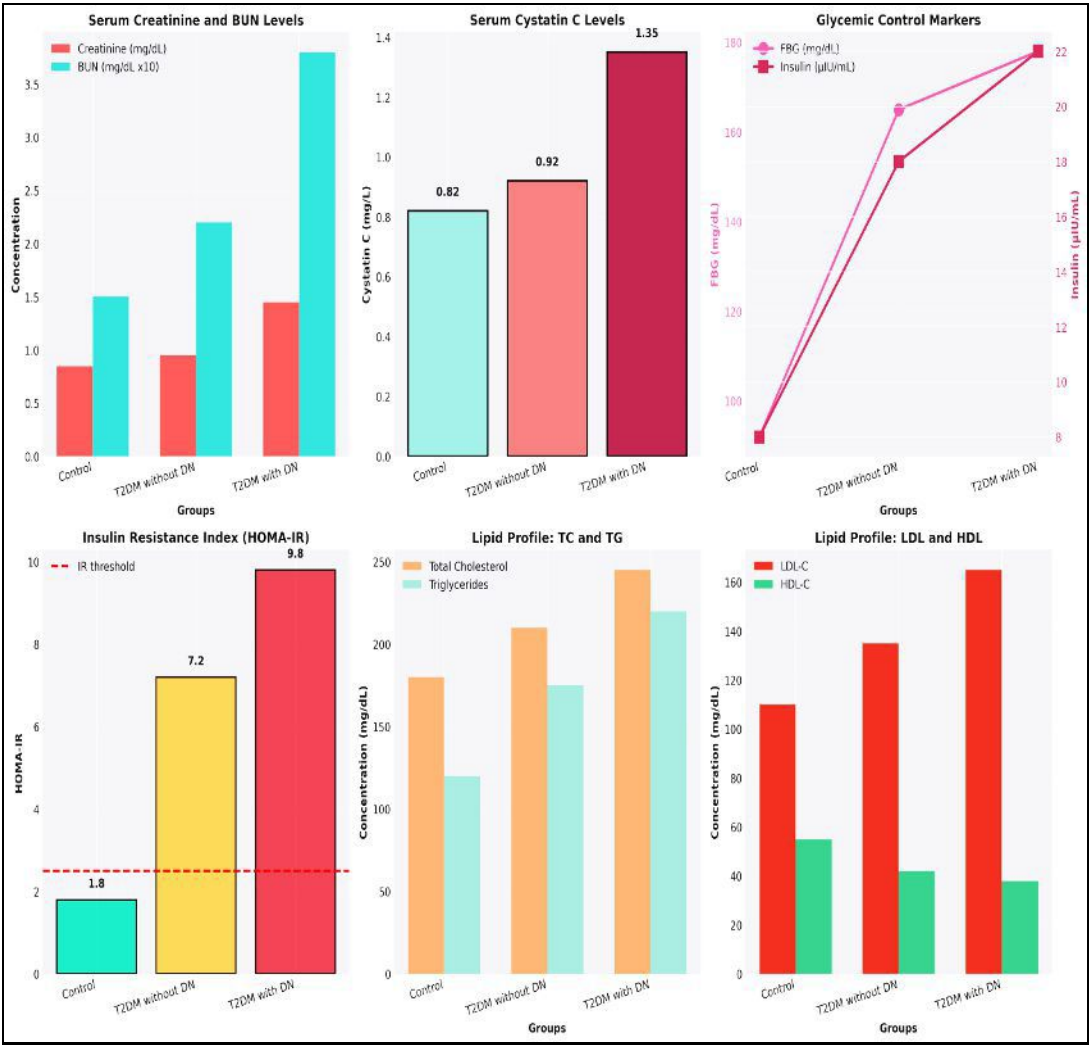


Fig 1: Comparative Analysis of Renal and Metabolic Biomarkers Across Study Groups This figure illustrates the progressive deterioration of renal function markers (creatinine, BUN, cystatin C), glycemic parameters (FBG, insulin), insulin resistance (HOMA-IR), and lipid profile (TC, TG, LDL, HDL) across control, T2DM without nephropathy, and T2DM with nephropathy groups.

Figure 2 shows the receiver operating characteristic (ROC) curve of cystatin C, serum creatinine and blood urea nitrogen (BUN) in detecting diabetic nephropathy. The diagnostic accuracy of cystatin C was the highest.

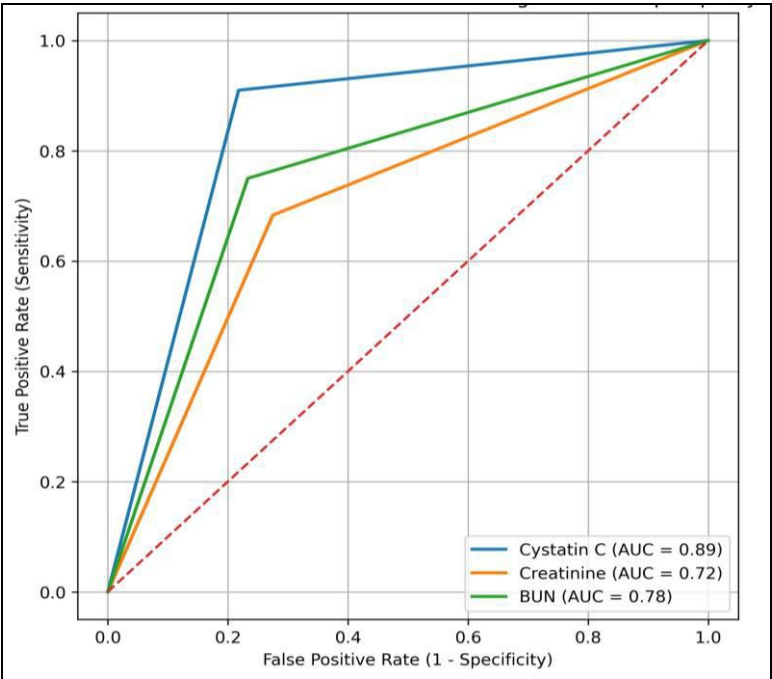


Fig 2: Roc curves of renal markers in DN patients

Discussion

Current comparative analysis provides a solid evidence that cystatin-C is a better early biomarker for diagnosis of DN as compared to conventional renal function markers, and it also shows the complex interrelationship between renal impairment, insulin resistance and dyslipidemia in T2DM subjects. This results have the potential to impact on clinical management and early intervention programmes [19, 20].

The above superiority of cystatin C over serum creatinine in detecting early stage renal impairment was also evident in our study, which showed cystatin C detected 91% positive cases while only 63% were detected by serum creatinine among patients with diabetic nephropathy [21]. This is consistent with other studies that have demonstrated cystatin C-based formulae can identify renal dysfunction before creatinine-based formulae [22]. The good diagnostic performance of cystatin C (AUC 0.89) in comparison with creatinine (AUC 0.72), also depends on independence from muscle mass, age, sex and dietary protein intake [23]. This study provides an additional basis for incorporating cystatin C assays into the regular screening of diabetic patients in a clinical setting, especially individuals at high risk of developing nephropathy.

The positive association between HOMA-IR and lipid abnormalities found in our study reflects the significance of IR in the metabolic perturbations associated with T2DM [24]. The incremental increase of HOMA-IR in study groups, up to 9.8 ± 3.1 for T2DM patients with nephropathy, illustrates the worsening metabolic milieu related to progressive renal disease. HOMA-IR is also significantly associated minute positive with the triglycerides ($r = 0.55$) and negative with HDL-C ($r = -0.45$) signifying that insulin resistance mediate the development of atherogenic dyslipidemia [25]. The present findings indicate that therapeutic strategies to combat insulin resistance may have concomitant effects to enhance glucose homeostasis and ameliorate lipid profiles.

The significant increase of BUN levels in patients with T2DM and nephropathy (38.5 ± 12.3 mg/dL) compared to those without nephropathy (22.1 ± 6.8 mg/dL) signifies a declined renal function [26]. Although BUN can be affected by factors such as dietary protein and hydration, its high correlation with other renal markers in our study supports it being an effective adjunctive biomarker. Analysis of multiple markers in aggregate is more robust, and has been shown to be superior to any single parameter [27].

Severe dyslipidemia in NDMM patients of T2DM-ND reflects the cytokine nexus because patients with diabetic nephropathy had significantly higher values for total cholesterol, triglycerides, LDL-C and VLDL-C but with reduced HDL-C [3]. These lipid disorders act not only as disease markers but also independently influence CVD risk and progression of nephropathy [28, 31]. The strong negative associations between eGFR and atherogenic lipid parameters (TC: $r = -0.42$; LDL-C: $r = -0.38$) indicate that renal dysfunction promotes dyslipidemia, which creates a vicious cycle to further advance both renal and cardiovascular events in progression.

The strengths of our study are that we conducted a in depth analysis with various classes of biomarkers performed using rigorous quality controls, and an adequate sample size with clearly defined patient groups. But limitations are cross-sectional design so that no temporal relationships or

causation can be interpreted [32-36]. Moreover, it was a single-center study, so there could be possible lack of generalization. Our findings should be validated in prospective longitudinal studies with larger, multicenter samples to identify whether intervention at an early stage based on cystatin C measurements can arrest progression to ESRD.

Conclusion

This comparative analysis shows cystatin C to be a more powerful marker than classical markers in the early recognition of diabetic nephropathy, with the latter revealing higher sensitivity (90.98%) and specificity (78.21%). Renal biomarkers (cystatin C, serum creatinine, BUN) when combined with metabolic indices (HOMAIR and lipid profile) are able to give a comprehensive stratification of risk in T2DM patients. The strong associations of insulin resistance and dyslipidemia with renal function impairment do, however, emphasize how closely metabolic and renal complications of diabetes are linked. The present findings provide additional support for cystatin C-based screening in clinical practice and have implications that emphasize the need for combined metabolic-renal therapy in order to prevent progression toward end-stage renal disease. The early detection of these biomarkers could aid in early intervention and prevent reduced long-term survival rates in T2DM.

References

1. Al-Karawi I, Majidi Z, Dashti N. Evaluating the role of vitamin D3 in modulating insulin resistance and glycemic control among type 2 diabetic nephropathy patients. *Acta Medica*. 2025;63(5):1-7.
2. Dejenie T, Abebe EC, Mengstie MA, Seid MA, Gebeyehu N, Adella GA, *et al*. Dyslipidemia and serum cystatin C levels as biomarker of diabetic nephropathy in patients with type 2 diabetes mellitus. *Front Endocrinol*. 2023;14:1124367.
3. Gembillo G, Sessa C, Santoro D. Cystatin C-based equations: Enhancing accuracy in kidney function tests for type 2 diabetes. *World J Nephrol*. 2025;14(3):102756.
4. Khalaf MS, Abdullah SA, Nasser N. A comparative study of cystatin C and creatinine as a preliminary marker of nephropathy in type 2 diabetic patients. *Eur J Med Health Res*. 2024;2(5):85-95.
5. Mahashabde ML, Chauhan RS, Paidi SKR, Sriram PJ. Study of cystatin C and renal resistive index in type 2 diabetes mellitus patients to detect early diabetic kidney disease. *Ann Afr Med*. 2025;24(2):235-242.
6. 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. *Life Sci*. 2024;5(1):41-47.
7. Gharib AF, Hassan AF, Saber T, Al-Qurashi SH, Al-Thobiti GM, Al-Tuwairqi EM, *et al*. Correlation of serum interleukin-7 with markers of insulin resistance in type 2 diabetes mellitus. *Clin Lab*. 2025;71(11):1-9.
8. Hadjari M, Bereksi K. Lipid profile, renal function tests and inflammatory markers in Algerian type 2 diabetic patients. *Iran J Public Health*. 2023;52(4):795-805.
9. Asmamaw T, Genet S, Menon M, Tarekegn G, Chekol E, Geto Z, *et al*. Early detection of renal impairment among patients with type 2 diabetes mellitus through

- evaluation of serum cystatin C in comparison with serum creatinine levels. *Diabetes Metab Syndr Obes.* 2020;13:4727-4738.
10. Boukrous H, Derghal R, Mezhoud I, Benaldjia H, Mallem NH, Djouimaa M, *et al.* Role of cystatin C and beta-2 microglobulin in the diagnosis of type 2 diabetic nephropathy. *J Biol Res.* 2025;98(1):1-10.
 11. Chait A, den Hartigh LJ. Adipose tissue distribution, inflammation and its metabolic consequences, including diabetes and cardiovascular disease. *Front Cardiovasc Med.* 2020;7:22.
 12. Cui L, Lu D, Tan S, Cao L. Comparative effectiveness of various combined interventions for type 2 diabetes and obesity: A systematic review and network meta-analysis. *Front Endocrinol.* 2025;16:1462104.
 13. Fatima A, Mal P, Memon ZR, Kumar M, Memon HJ, Fatima M. Evaluation of serum cystatin C as a predictor of eGFR in type 2 diabetic patients with nephropathy. *J Shaheed Mohtarma Benazir Bhutto Med Coll.* 2025;6(1):1-6.
 14. Grundy SM, Benjamin IJ, Burke GL, Chait A, Eckel RH, Howard BV, *et al.* Diabetes and cardiovascular disease: A statement for healthcare professionals from the American Heart Association. *Circulation.* 1999;100(10):1134-1146.
 15. Hameed SM, Abdulrahman SJ. Evaluation of the role of lipid profile and kidney function in men with type 2 diabetes in the city of Kirkuk. *Int J Life Sci Res Arch.* 2025;8(2):276-285.
 16. Hu H, Nakagawa T, Honda T, Yamamoto S, Mizoue T. Should insulin resistance, insulin secretion and visceral fat area be considered for improving diabetes risk prediction models? *BMJ Open Diabetes Res Care.* 2024;12(1):e003680.
 17. Iacu CO, Steen A, Cova M. Gut microbiota and complications of type-2 diabetes. *Nutrients.* 2022;14(1):166.
 18. Jawad H, Ali S, Jawad M. Impact of serum adiponectin level on insulin and hs-CRP in patients with type 2 diabetes. *Ann Surg Innov Dev Endosc Interv Med.* 2025;3(2):1-8.
 19. Lin SZ, Kuo W, Tsai BCK, Paul CR, Kuo CH, Hsieh D, *et al.* Therapeutic potential of *Cordyceps militaris* cultivated with *Ginkgo biloba* seeds for alleviating western diet-induced type 2 diabetes and diabetic nephropathy. *Front Pharmacol.* 2025;16:1562116.
 20. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, *et al.* Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology.* 2003;37(4):917-923.
 21. Perkovic V, Jardine M, Neal B, Bompoint S, Heerspink HJL, Charytan D, *et al.* Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N Engl J Med.* 2019;380(24):2295-2306.
 22. Sang D, Tao J, Song W, Zhang Q, Wu S, Geng W. Comparison of creatinine-and cystatin C-based eGFR and their association with heart failure in type 2 diabetes. *Diabetol Metab Syndr.* 2024;16:225.
 23. Wu Z, Zhang B, Chen F, Xia R, Zhu D, Chen B, *et al.* Fecal microbiota transplantation reverses insulin resistance in type 2 diabetes. *Front Cell Infect Microbiol.* 2023;12:1089991.
 24. Yahya A, Kadhimi D, Abdalhadi N. Kidney injury molecule-1 and cystatin C as early biomarkers for renal dysfunction in Iraqi type 2 diabetes mellitus patients. *J Appl Biotechnol Bioeng.* 2023;10(6):195-201.