Indonesian Journal of Cardiology
Indonesian J Cardiol • Vol. 47 • 2026
pISSN: 2830-3105 / eISSN: 2964-7304
doi: 10.30701/ijc.2105

Original Article

Multimarker Assessment of Cardiovascular Risk in Type 2 Diabetes: Role of Inflammation, Myocardial Injury, and Glycaemic Control

Atul Khajuria[1], Gagandeep Singh[2]

Abstract

Background: Diabetes mellitus, particularly Type 2 Diabetes (T2DM), has become a major public health concern globally, especially in low- and middle-income countries like India. The prevalence of T2DM in India is rising, with Punjab showing particularly high rates due to dietary patterns, sedentary lifestyles, and genetic predisposition. T2DM is strongly linked to Cardiovascular Disease (CVD), and inflammatory markers such as high-sensitivity C-Reactive Protein (hs-CRP), fibrinogen, and Hemoglobin A1c (HbA1c) may be associated with higher cardiovascular risk in diabetic patients. This study aims to evaluate the role of hs-CRP, fibrinogen, HbA1c, and other biomarkers in assessing cardiovascular risk in patients with T2DM in Punjab, North India, and to examine whether these biomarkers show additional associations with higher cardiovascular risk strata beyond traditional markers like Low-Density Lipoprotein Cholesterol (LDL-C).

Methods: A cross-sectional observational study was conducted from January 2024 to June 2025, involving 1,200 patients diagnosed with T2DM from both urban and rural areas of Punjab. Participants aged 30–70 years were selected using stratified random sampling. Data collection included demographic, clinical, and biochemical information. Fasting blood samples were analyzed for hs-CRP, fibrinogen, HbA1c, LDL-C, and other biomarkers. Cardiovascular risk was assessed using the Framingham Risk Score (FRS), which, in this South Asian diabetic cohort, was applied as a comparative risk stratification tool rather than as a fully validated prognostic model. Multivariable logistic regression and Receiver Operating Characteristic (ROC) curve analysis were used to assess associations between biomarkers and higher cardiovascular risk categories and the presence of CVD.

Results: The study found significant differences in biomarker levels between urban and rural populations, with rural participants showing higher levels of hs-CRP, fibrinogen, HbA1c, LDL-C, and other markers of myocardial injury, including troponins and B-type Natriuretic Peptide (BNP). Elevated hs-CRP (>3 mg/L), fibrinogen (>400 mg/dL), and HbA1c (>9%) were associated with higher FRS-defined cardiovascular risk categories and with CVD presence, and logistic regression identified hs-CRP and LDL-C as independently associated with CVD after adjustment for conventional risk factors. Biomarkers such as High-Sensitive Troponin I (hs-TnI) and Lipoprotein-associated Phospholipase A2 (Lp-PLA2) were also strongly associated with prevalent CVD.

Conclusions: This cross-sectional study highlights that inflammatory and cardiac biomarkers are strongly associated with higher cardiovascular risk strata and prevalent CVD in T2DM patients, particularly in rural populations where biomarker levels and calculated risk scores are higher. The findings support the potential role of these biomarkers in multimarker risk stratification in diabetic populations, especially in resource-limited settings, while underscoring that longitudinal studies are required before inferring prognostic or predictive value or implementing biomarker-guided interventions.

Keywords: Type 2 Diabetes Mellitus, Cardiovascular Risk, hs-CRP, Fibrinogen, Biomarkers, Framingham Risk Score, Punjab, Urban vs Rural, CVD Risk Stratification, Inflammation, Glycemic Control

[1]Dean, Allied & Healthcare Sciences, Rayat Bahra Professional University, Hoshiarpur–Chandigarh Road, VPO Bohan, Hoshiarpur, Punjab, India.
[2]Assistant Professor, Faculty of Allied Healthcare Sciences, Desh Bhagat University, Mandi Gobindgarh, Punjab, India.

Correspondence:
Atul Khajuria,

Dean, Allied & Healthcare Sciences, Rayat Bahra Professional University, Hoshiarpur–Chandigarh Road, VPO Bohan, Hoshiarpur, Punjab, India.

Email: atulkhajuria83@gmail.com

Introduction

Diabetes mellitus, particularly Type 2 Diabetes (T2DM), has emerged as one of the most significant public health challenges worldwide. The increasing prevalence of diabetes in low- and middle-income countries, including India, is driven by rapid urbanization, lifestyle changes, and dietary habits. In India alone, an estimated 77 million adults were living with diabetes in 2023, with the numbers expected to rise further in the coming decades.[1] Among Indian states, Punjab stands out for its high diabetes prevalence, a consequence of dietary patterns rich in carbohydrates and fats, sedentary lifestyles, and the genetic predisposition of its population. This trend is alarming given the limited healthcare resources in many areas, particularly in rural regions.

The impact of diabetes on cardiovascular health is well-documented. Cardiovascular Disease (CVD) is the leading cause of mortality in diabetic individuals, with studies showing that diabetes increases the risk of coronary artery disease, heart failure, and stroke. Diabetic patients are at higher risk of these conditions due to the chronic hyperglycemia and associated metabolic disturbances that contribute to endothelial dysfunction, a key driver of atherosclerosis. However, current cardiovascular risk assessments are largely based on traditional markers such as lipid profiles, blood pressure, and glycaemic control. These markers, while important, do not fully reflect the complexity of cardiovascular risk in diabetic patients, particularly the inflammatory processes that drive the progression of atherosclerosis and other cardiovascular events.

The role of inflammation in CVD has gained increasing recognition in recent years. Inflammatory markers, such as high-sensitivity C-Reactive Protein (hs-CRP), fibrinogen, and interleukin-6, have been shown to be independently associated with cardiovascular events in longitudinal studies. Elevated levels of hs-CRP, in particular, have been associated with increased risk of heart attacks, strokes, and other atherosclerotic events, even in individuals with normal cholesterol levels.[3-4] Hs-CRP is an acute-phase reactant produced by the liver in response to systemic inflammation and has been shown to provide incremental value in cardiovascular risk stratification in multiple cohorts, beyond what is offered by traditional lipid-based assessments.[5] In fact, studies such as the Justification for the Use of Statins in Prevention (JUPITER) trial have demonstrated that statin therapy, aimed at reducing hs-CRP levels, can significantly lower cardiovascular event rates, even in individuals without elevated cholesterol levels.[5]

The utility of hs-CRP for cardiovascular risk stratification has not been extensively studied in diabetic populations, particularly in regions such as Punjab, where diabetes is highly prevalent. Moreover, while hs-CRP has been widely studied in Western populations, there is limited data on how this marker performs in the context of Indian patients with diabetes, where genetic, environmental, and lifestyle factors may differ significantly from those in other parts of the world.

Beyond hs-CRP, other biomarkers such as fibrinogen and HbA1c also have established roles in cardiovascular risk assessment. Fibrinogen is a key protein in the coagulation pathway and has been associated with increased blood clot formation and atherosclerotic plaque instability. Elevated fibrinogen levels have been shown to predict cardiovascular events, particularly in individuals with diabetes.[6] Similarly, HbA1c, a marker of long-term glycaemic control, remains a cornerstone of diabetes management, and its relationship with cardiovascular risk is well documented. However, these biomarkers have typically been studied in isolation, and there is a need for research that evaluates their combined role in cardiovascular risk stratification. This study aims to evaluate the role of hs-CRP and other biomarkers, including lipid profile, HbA1c, and fibrinogen, in assessing cardiovascular risk in patients with T2DM in Punjab, North India. This study aims to evaluate whether hs-CRP and related biomarkers show additional associations with higher cardiovascular risk categories and prevalent CVD, beyond traditional biomarkers such as Low-Density Lipoprotein Cholesterol (LDL-C) and HbA1c, in adults with T2DM in Punjab.

Methods

Study Design

This study is a cross-sectional, observational research conducted between January 2024 and June 2025 in Punjab, North India. The study aimed to evaluate the role of inflammatory and metabolic biomarkers, including hs-CRP, lipid profile, HbA1c, and fibrinogen, in assessing cardiovascular risk in diabetic patients. A stratified random sampling technique was used to recruit participants from both urban and rural healthcare centers in Punjab to ensure representation from both populations. Due

to the cross-sectional design, exposure (biomarker) and outcome (CVD status and FRS category) were assessed at the same time, so only associations—not causal or prognostic relationships—can be inferred.

Study Population

A total of 1,200 patients diagnosed with T2DM were recruited for the study. Participants were aged between 30 and 70 years. The inclusion and exclusion criteria were strictly followed to ensure the selection of a homogenous study population that represented typical diabetic patients in this region.

Inclusion Criteria

- Diagnosis of T2DM for at least one year.
- Age between 30 and 70 years.
- Willingness to provide informed consent to participate in the study.

Exclusion Criteria

- Diagnosis of type 1 diabetes mellitus.
- History of autoimmune diseases or chronic inflammatory conditions (e.g., rheumatoid arthritis, lupus).
- Recent history of acute infections, surgeries, or trauma within the past three months.
- Pregnancy or lactation.
- Current use of immunosuppressive or anti-inflammatory medications.

Ethical Approval

The study was approved by the Institutional Ethics Committee of Rayat Bahra Professional University, and written informed consent was obtained from all participants before their inclusion in the study. All procedures were conducted in compliance with the Declaration of Helsinki and ethical guidelines for human research.

Data Collection

Demographic and Clinical Data

- Demographic details, including age, sex, Body Mass Index (BMI), and smoking status, were recorded using a structured questionnaire.
- Clinical data, information on current cardiovascular and diabetes medications was extracted from prescriptions and medical records, including statins, antihypertensive agents (e.g., Angiotensin-Converting Enzyme (ACE) inhibitors/Angiotensin II Receptor Blockers (ARBs), beta-blockers, calcium channel blockers, diuretics), and glucose-lowering therapies (oral agents and insulin). Medication use was coded as categorical variables (yes/no for each major drug class) were obtained from medical records and patient interviews.

Biochemical Analysis

Fasting blood samples were collected for biomarker assessment were obtained at the time of study enrolment, after stabilization of the patients' usual outpatient status. Patients with an acute coronary or cerebrovascular event within the preceding three months, or with acute infection, surgery, or trauma in the past three months, were excluded to minimize acute-phase effects on biomarker levels. These samples were used for the following biochemical analyses:

- High-sensitivity C-reactive protein (hs-CRP)

  Serum hs-CRP was quantified using the Abbott Architect i2000SR immunoassay system with the Abbott hs-CRP reagent kit, in accordance with the manufacturer's protocols. The assay was performed as a Chemiluminescent Microparticle Immunoassay (CMIA), with results reported in mg/L.

  Cutoff Values: hs-CRP levels were categorized according to established guidelines, with <1.0 mg/L indicating low cardiovascular risk, 1.0–3.0 mg/L indicating intermediate risk, and >3.0 mg/L indicating high risk. Levels exceeding 10 mg/L typically suggest acute inflammation, necessitating further clinical investigation.
- Lipid profile: Lipid Profile Measurement

  Total cholesterol (TC), LDL-C, High-Density Lipoprotein Cholesterol (HDL-C), and Triglycerides (TG) were measured in serum using Abbott enzymatic assays on the Abbott Architect i2000SR system. The assays utilize enzymatic colorimetric methods to quantify lipid fractions with spectrophotometric detection.

  Cutoff Values:
  - Total Cholesterol (TC): <200 mg/dL (desirable), ≥240 mg/dL (high risk).
  - LDL-C: <100 mg/dL (optimal), ≥160 mg/dL (high risk).
  - HDL-C: <40 mg/dL (low risk), ≥60 mg/dL (protective).
  - TG: <150 mg/dL (normal), ≥150 mg/dL (borderline/high).
- Glycated Hemoglobin (HbA1c) Measurement
- HbA1c was measured in EDTA whole blood using High Performance Liquid Chromatography (HPLC) on the Bio Rad Variant II Turbo System with the appropriate HbA1c reagent kit, as per the manufacturer's instructions. The assay separates hemoglobin

fractions based on charge differences, and results were expressed as % HbA1c (National Glycohemoglobin Standardization Program [NGSP]). Calibration was performed according to the kit insert, and daily quality controls at two levels were analyzed to ensure methodological precision and accuracy.

Cutoff Values: HbA1c was categorized per standard diagnostic thresholds: <5.7% (normal), 5.7–6.4% (prediabetes), and ≥6.5% (diabetes). These cutoffs are widely accepted and have been validated in Indian adult populations in multiple studies.

- Fibrinogen: Plasma Fibrinogen Measurement

  Plasma fibrinogen was measured using the Abbott ACL TOP Analyzer with the Abbott fibrinogen reagent kit, following the manufacturer's instructions. This clot-based assay measures fibrinogen concentration by determining the clotting time of plasma after the addition of reagent, with the time inversely related to fibrinogen levels. Results were expressed in g/L.

  Plasma fibrinogen levels were categorized as follows:
  - Normal Range: 2.0–4.0 g/L
  - Elevated Fibrinogen: >4.0 g/L, associated with increased cardiovascular and thrombotic risk. These cutoffs are consistent with both global guidelines and Indian studies linking elevated fibrinogen with metabolic syndrome, diabetes, and cardiovascular risk.
- Renal function

  Renal function was assessed using the Abbott Architect i2000SR analyzer with Abbott enzymatic reagent kits for serum creatinine, blood urea (BUN/urea), and calculation of estimated Glomerular Filtration Rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Results were reported in mg/dL (creatinine and urea) and mL/min/1.73 m² (eGFR). Calibration was performed with Abbott standard calibrators, and daily internal quality controls were run to ensure accuracy and precision.

  Cutoff Values:
  - Serum Creatinine (Men): 0.6–1.2 mg/dL, (Women): 0.5–1.1 mg/dL
  - Blood Urea (BUN/urea): 7–20 mg/dL
  - eGFR (Normal): ≥90 mL/min/1.73 m², (Mildly Reduced): 60–89 mL/min/1.73 m², (CKD): <60 mL/min/1.73 m²
  - Urea-to-Creatinine Ratio: 10:1 to 20:1

  These values align with established global and Indian reference ranges, with creatinine >1.2 mg/dL and eGFR <60 mL/min/1.73 m² suggesting renal impairment and early-stage CKD.

Biomarker Measurement

Biomarkers were measured using the Abbott Architect i2000SR system with Abbott CLIA reagent kits for troponins (hs-cTnI, hs-cTnT), BNP/N-Terminal pro-B-type Natriuretic Peptide (NT-proBNP), D-dimer, Lipoprotein-associated Phospholipase A2 (Lp-PLA2), Creatine Kinase-MB (CK-MB), and myoglobin/Heart-Type Fatty Acid-Binding Protein (H-FABP). All assays utilized CMIA technology. Calibration was performed with Abbott standard calibrators, and daily internal quality controls were used to ensure precision.

Cutoff Values:
- hs-cTnI (female): <16 ng/L, (male): <34 ng/L
- BNP: <100 pg/mL, NT-proBNP: <300 pg/mL
- D-dimer: <500 ng/mL FEU
- Lp-PLA2: <225 nmol/min/mL
- CK-MB: <5 ng/mL
- Myoglobin: <70 ng/mL, H-FABP: <7 ng/mL

Cardiovascular Risk Assessment

- Framingham Risk Score (FRS)

  Cardiovascular risk was assessed using the FRS, a widely used tool that estimates 10-year risk of cardiovascular events based on age, gender, cholesterol levels, blood pressure, smoking status, and diabetes. The FRS was originally developed in predominantly Western, mixed-risk populations and has limited validation in South Asian cohorts with established diabetes. In this study, the Framingham Total CVD Risk Score was calculated for each participant and used primarily as a comparative risk stratification framework to classify individuals into relative low, moderate, and high risk categories within the cohort, rather than as a fully validated tool for estimating absolute 10-year event probabilities.
- Incremental Value of Biomarkers

  The study also aimed to evaluate the incremental association of hs-CRP, fibrinogen, and HbA1c with higher FRS-defined risk categories and prevalent CVD, after adjustment

for traditional risk factors. Logistic regression models were used to examine the relationship between these biomarkers and cardiovascular risk, adjusting for potential confounders such as age, gender, and BMI.

Statistical Analysis

For some analyses, continuous biomarker values were categorized using clinically and guideline-based cut-offs (e.g., hs-CRP <1.0, 1.0–3.0, >3.0 mg/L; fibrinogen >4.0 g/L; LDL-C ≥130 mg/dL; BNP ≥150 pg/mL; hs-TnI ≥10 ng/L), reflecting thresholds used in prior cardiovascular and diabetes studies and facilitating clinical interpretation. We acknowledge that categorizing continuous variables can lead to loss of information and reduced statistical power; therefore, key biomarkers were also modeled as continuous predictors in multivariable logistic regression and Receiver Operating Characteristic (ROC) analyses, which yielded similar directions and magnitudes of association

Data Processing

Data were entered into a statistical software package (SPSS v26.0). Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables were presented as frequencies and percentages.

Univariate Analysis

- Differences in continuous variables between groups were assessed using independent t-tests or ANOVA
- Differences in categorical variables were analysed using the chi-square test.

Multivariate Logistic Regression

To assess the association between biomarkers and cardiovascular risk, multivariate logistic regression models were constructed. These models were adjusted for known cardiovascular risk factors such as age, sex, BMI, and smoking status. The Odds Ratio (OR) and 95% Confidence Intervals (CIs) were calculated to estimate the strength of the associations.

Receiver Operating Characteristic (ROC) Curve Analysis

To evaluate the diagnostic accuracy of hs-CRP and other biomarkers, ROC curves were generated. The Area Under the Curve (AUC) was used to assess the ability of these biomarkers to discriminate between individuals with high and low cardiovascular risk.

Subgroup Analysis

A subgroup analysis was conducted to assess differences in biomarker levels and cardiovascular risk between urban and rural participants, as there are known disparities in healthcare access and lifestyle factors between these groups in Punjab.

Continuous Biomarker

Continuous biomarker values were categorized using clinically and guideline-based cut-offs (e.g., hs-CRP <1.0, 1.0–3.0, >3.0 mg/L; fibrinogen >4.0 g/L; LDL-C ≥130 mg/dL; BNP ≥150 pg/mL; hs-TnI ≥10 ng/L), reflecting thresholds used in prior cardiovascular and diabetes studies and facilitating clinical interpretation. We acknowledge that categorizing continuous variables can lead to loss of information and reduced statistical power; therefore, key biomarkers were also modeled as continuous predictors in multivariable logistic regression and ROC analyses, which yielded similar directions and magnitudes of association

Results

A total of 1,200 diabetic patients participated in the study, with a mean age of 52.3 ± 10.4 years. The sample included 660 males (55%) and 540 females (45%). Of the participants, 60% were from urban areas, and 40% were from rural areas. Baseline characteristics, including BMI, Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), and smoking status, were recorded. The mean BMI was 27.4 ± 4.3 kg/m², indicating that the majority of the participants were overweight. The average SBP and DBP were 136 ± 18 mmHg and 84 ± 12 mmHg, respectively.

Table 1 presents demographic and health-related characteristics of the study population, divided into urban and rural subgroups. The average age and BMI are similar across both groups, while rural participants show slightly higher diastolic blood pressure, reflecting modest differences without implying causality.

Table 2 shows that the Rural participants demonstrated a consistently less favorable biomarker profile than urban participants. Mean hs CRP was high in the total sample (4.2 ± 1.3 mg/L) and significantly higher in rural than urban residents (3.1 ± 1.3 vs. 2.8 ± 1.1 mg/L; $p < 0.001$), indicating greater systemic inflammation in the rural group. Glycaemic and lipid control were also poorer in rural participants, with slightly higher HbA1c (8.4 ± 1.5% vs. 8.1 ± 1.3%; $p = 0.02$) and higher LDL-C (142 ± 33 vs. 136 ± 31 mg/dL; $p = 0.02$), while HDL-C was modestly lower but not significantly different ($p = 0.07$). Markers of myocardial injury and cardiac strain, including Troponin I, Troponin T, hs TnI, hs TnT, CK MB, Myoglobin/H FABP,

BNP and NT proBNP, were all significantly higher in rural compared with urban participants (all $p \leq 0.04$), suggesting greater subclinical myocardial damage and hemodynamic stress in the rural subgroup. In addition, D-dimer and Lp-PLA2 levels were higher in rural participants ($p = 0.02$ and $p < 0.01$, respectively), pointing to increased coagulation activity and vascular inflammatory burden in this population.

Table 3 compares biomarker levels across total, urban, and rural populations, showing significant regional differences. Rural populations generally have higher levels of biomarkers, including hs-CRP (3.6 ± 1.4 vs. 2.8 ± 1.1 in urban), fibrinogen (405 ± 74 vs. 392 ± 65), HbA1c (8.4 ± 1.5 vs. 8.1 ± 1.3), and LDL-C (142 ± 33 vs. 136 ± 31), with p-values indicating statistical significance. Markers like Troponin-I, Troponin-T, and CK-MB also show higher levels in rural populations, reflecting potential cardiovascular risks. Other biomarkers, such as BNP, NT-proBNP, D-Dimer, and Lp-PLA2, also demonstrate higher values in rural areas. These findings suggest differences in health conditions or lifestyle factors between rural and urban populations.

Table 4 presents a comparison of various health biomarkers between urban and rural populations, revealing notable differences, particularly for inflammation, glycemic control, cardiovascular risk, and myocardial injury. hs-CRP (mg/L), a marker of inflammation, was significantly higher in rural populations (3.6 ± 1.4) compared to urban populations (2.8 ± 1.1), with a p-value of 0.03,

Table 1. Baseline characteristics of study population.

Characteristic	Total (N=1200)	Urban (N=720)	Rural (N=480)
Age (years)	52.3 ± 10.4	52.0 ± 10.3	52.7 ± 10.6
Gender (Male)	660 (55%)	380 (53%)	280 (58%)
BMI (kg/m²)	27.4 ± 4.3	27.2 ± 4.1	27.7 ± 4.5
Systolic BP (mmHg)	136 ± 18	137 ± 19	134 ± 16
Diastolic BP (mmHg)	84 ± 12	83 ± 11	86 ± 13

BMI: Body Mass Index; BP: Blood Pressure.

Table 2. Biomarker levels and cardiovascular risk.

Biomarker	Total	Urban	Rural	p-value
hs-CRP (mg/L)	3.1 ± 1.3	2.8 ± 1.1	3.6 ± 1.4	<0.001
Fibrinogen (mg/dL)	398 ± 68	392 ± 65	405 ± 74	0.04
HbA1c (%)	8.2 ± 1.4	8.1 ± 1.3	8.4 ± 1.5	0.02
LDL Cholesterol (mg/dL)	138 ± 32	136 ± 31	142 ± 33	0.02
HDL Cholesterol (mg/dL)	40 ± 12	41 ± 13	39 ± 11	0.07
Troponin-I (ng/mL)	0.034 ± 0.02	0.031 ± 0.02	0.038 ± 0.02	0.03
Troponin-T (ng/mL)	0.016 ± 0.01	0.014 ± 0.01	0.018 ± 0.01	0.04
hs-TnI (ng/L)	7.8 ± 3.2	7.2 ± 3.1	8.6 ± 3.4	<0.01
hs-TnT (ng/L)	11.4 ± 4.1	10.9 ± 3.8	12.1 ± 4.4	0.02
CK-MB (ng/mL)	16.5 ± 4.8	15.8 ± 4.5	17.4 ± 4.9	<0.01
Myoglobin/H-FABP (ng/mL)	48.6 ± 15.4	45.3 ± 14.8	52.7 ± 16.1	<0.01
BNP (pg/mL)	118 ± 40	112 ± 38	126 ± 41	<0.01
NT-proBNP (pg/mL)	186 ± 52	178 ± 50	197 ± 54	<0.001
D-Dimer (mg/L)	0.62 ± 0.28	0.58 ± 0.27	0.68 ± 0.29	0.02
Lp-PLA2 (ng/mL)	208 ± 35	202 ± 33	216 ± 36	<0.01

hs-CRP: high-sensitivity C-Reactive Protein; HbA1c: Hemoglobin A1c; LDL-C: Low-Density Lipoprotein Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; hs-TnI: High-Sensitive Troponin I; hs-TnT: High-Sensitive Troponin T; CK-MB: Creatine Kinase-MB; H-FABP: Heart-Type Fatty Acid-Binding Protein; BNP: B-type Natriuretic Peptide; NT-proBNP: N-Terminal pro-B-type Natriuretic Peptide; Lp-PLA2: Lipoprotein-associated Phospholipase A2.

Table 3. Association between biomarkers and CVD risk.

Biomarker	Threshold	OR	95% CI	p-value
hs-CRP	>3 mg/L	2.5	1.8-3.4	<0.001
Fibrinogen	>400 mg/dL	1.8	1.4-2.3	<0.001
HbA1c	Each 1% ↑	1.16	1.08-1.25	<0.01
LDL-C	>130 mg/dL	2.1	1.6-2.7	<0.001
Troponin-I	>0.04 ng/mL	3.0	2.1-4.0	<0.001
hs-TnI	>10 ng/L	3.6	2.5-4.7	<0.001
CK-MB	>20 ng/mL	2.4	1.6-3.2	<0.001
BNP	>150 pg/mL	2.8	2.0-3.7	<0.001
D-Dimer	>0.7 mg/L	1.9	1.4-2.6	<0.01
Lp-PLA2	>220 ng/mL	2.2	1.6-2.9	<0.001
High LDL-C + High hs-CRP	Combined	3.3	2.5-4.4	<0.001

hs-CRP: high-sensitivity C-Reactive Protein; HbA1c: Hemoglobin A1c; LDL-C: Low-Density Lipoprotein Cholesterol; hs-TnI: High-Sensitive Troponin I; CK-MB: Creatine Kinase-MB; BNP: B-type Natriuretic Peptide; Lp-PLA2: Lipoprotein-associated Phospholipase A2.

Table 4. Urban vs. rural comparison.

Variable	Urban	Rural	p-value	Comments
hs-CRP (mg/L)	2.8 ± 1.1	3.6 ± 1.4	0.03	Higher inflammation in rural
HbA1c (%)	8.1 ± 1.3	8.4 ± 1.5	0.02	Poorer glycemic control rural
FRS (%)	17.1 ± 4.9	19.3 ± 5.7	<0.001	Higher CVD risk rural
Troponin-I	7.2 ng/L	8.6 ng/L	<0.01	Higher myocardial injury rural
BNP (pg/mL)	112	126	<0.01	Higher cardiac strain rural
D-Dimer	0.58	0.68	0.02	Higher coagulation risk

hs-CRP: high-sensitivity C-Reactive Protein; HbA1c: Hemoglobin A1c; FRS: Framingham Risk Score; BNP: B-type Natriuretic Peptide.

indicating higher inflammation levels in rural areas. Similarly, HbA1c (%), a measure of long-term blood sugar control, was also higher in rural populations (8.4 ± 1.5) compared to urban populations (8.1 ± 1.3), suggesting poorer glycemic control in rural areas ($p = 0.02$). The FRS, which estimates the risk of CVD, was higher in rural populations (19.3 ± 5.7) than in urban populations (17.1 ± 4.9), with a p-value of <0.001, indicating a higher cardiovascular risk in rural areas. Troponin-I, a marker of myocardial injury, was also elevated in rural populations (8.6 ng/L) compared to urban populations (7.2 ng/L), with a significant p-value of <0.01, reflecting higher myocardial injury risk in rural individuals. Similarly, BNP (pg/mL), a marker of cardiac strain, was higher in rural populations (126) than urban populations (112), with a p-value of <0.01, further indicating greater cardiac strain in rural areas.

D-Dimer, a marker of coagulation risk, was significantly higher in rural populations (0.68) compared to urban populations (0.58), with a p-value of 0.02, suggesting an increased risk of blood clot formation in rural areas. Overall, these findings suggest that rural populations may be at higher risk for inflammation, poor glycaemic control, CVD, myocardial injury, cardiac strain, and coagulation issues compared to urban populations.

Table 5 highlights biomarker levels across low, moderate, and high-risk groups, with significant differences ($p < 0.001$) observed in each marker. For hs-CRP (mg/L), levels increased from 2.1 ± 1.0 in the low-risk group to 3.8 ± 1.5 in the high-risk group. Fibrinogen (mg/dL) levels were 380 ± 70 in low-risk, 395 ± 68 in moderate-risk, and 425 ± 90 in high-risk groups. HbA1c (%) showed an increase from 7.8 ± 1.2 in low-risk to 8.9 ± 1.5 in high-risk. LDL-C (mg/dL) levels rose from 128 ± 28 in low-risk to 150 ± 36 in high-risk groups. For hs-TnI (ng/L), levels increased from 6.2 ± 2.4 in the low-risk group to 10.5 ± 4.2 in the high-risk group. BNP (pg/mL) showed a progression from 102 ± 35 in low-risk to 155 ± 42 in high-risk groups.

Finally, Lp-PLA2 (ng/mL) levels increased from 192 ± 30 in low-risk to 236 ± 40 in high-risk groups. These findings indicate a clear trend of worsening biomarker levels with increasing risk, reflecting higher inflammation, poor glycemic control, and greater cardiovascular risk.

Table 6 reveals that the Participants with CVD showed a markedly higher prevalence of high risk biomarker categories than those without CVD. High hs CRP was present in 62.5% of CVD cases versus 31.25% of non CVD participants, and high fibrinogen in 37.5% versus 12.5%. Poor glycaemic control (HbA1c >9%) and elevated LDL-C (>130 mg/dL) were also more frequent in CVD (30% and 55%) than non CVD (12.5% and 22.5%). Similarly, elevated hs TnI, CK MB, BNP and D dimer occurred roughly two to three fold more often among CVD cases, indicating strong clustering of inflammatory, metabolic, myocardial injury and pro thrombotic abnormalities with CVD.

Table 5. ANOVA-Biomarkers across risk categories.

Biomarker	Low Risk	Moderate	High Risk	p-value
hs-CRP (mg/L)	2.1 ± 1.0	2.7 ± 1.2	3.8 ± 1.5	<0.001
Fibrinogen (mg/dL)	380 ± 70	395 ± 68	425 ± 90	<0.001
HbA1c (%)	7.8 ± 1.2	8.3 ± 1.4	8.9 ± 1.5	<0.001
LDL-C (mg/dL)	128 ± 28	137 ± 32	150 ± 36	<0.001
hs-TnI (ng/L)	6.2 ± 2.4	8.1 ± 3.1	10.5 ± 4.2	<0.001
BNP (pg/mL)	102 ± 35	125 ± 38	155 ± 42	<0.001
Lp-PLA2 (ng/mL)	192 ± 30	210 ± 32	236 ± 40	<0.001

hs-CRP: high-sensitivity C-Reactive Protein; HbA1c: Hemoglobin A1c; LDL-C: Low-Density Lipoprotein Cholesterol; hs-TnI: High-Sensitive Troponin I; BNP: B-type Natriuretic Peptide; Lp-PLA2: Lipoprotein-associated Phospholipase A2.

Table 6. Biomarker category vs. CVD presence.

Variable	CVD present (N = 480) Rural	CVD absent (N = 720), Urban	p value
High hs-CRP (≥3 mg/L)	62.5% (300/480)	31.25% (225/720)	<0.001
High fibrinogen	37.5% (180/480)	12.5% (90/720)	<0.001
HbA1c >9%	30% (144/480)	12.5% (90/720)	<0.001
LDL-C >130 mg/dL	55% (264/480)	22.5% (162/720)	<0.001
hs TnI >10 ng/L	48% (230/480)	18% (130/720)	<0.001
CK-MB >20 ng/mL	34% (163/480)	14% (101/720)	<0.001
BNP >150 pg/mL	42% (202/480)	15% (108/720)	<0.001
D dimer >0.7 mg/L	29% (139/480)	11% (80/720)	<0.001

hs-CRP: high-sensitivity C-Reactive Protein; HbA1c: Hemoglobin A1c; LDL-C: Low-Density Lipoprotein Cholesterol; hs-TnI: High-Sensitive Troponin I; CK-MB: Creatine Kinase-MB; BNP: B-type Natriuretic Peptide.

Table 7. Logistic regression for CVD prediction.

Biomarker	OR	95% CI	p-value
hs-CRP	2.5	1.8-3.4	<0.001
Fibrinogen	1.8	1.4-2.3	<0.001
HbA1c	1.16	1.08-1.25	<0.01
LDL-C	2.1	1.6-2.7	<0.001
hs-TnI	3.4	2.4-4.5	<0.001
BNP	2.6	1.9-3.4	<0.001
Lp-PLA2	2.0	1.5-2.7	<0.001

hs-CRP: high-sensitivity C-Reactive Protein; HbA1c: Hemoglobin A1c; LDL-C: Low-Density Lipoprotein Cholesterol; hs-TnI: High-Sensitive Troponin I; BNP: B-type Natriuretic Peptide; Lp-PLA2: Lipoprotein-associated Phospholipase A2.

Table 7 shows the OR and confidence intervals (CI) for various biomarkers linked to a health outcome. hs-CRP (OR 2.5) and LDL-C (OR 2.1) both show strong associations, indicating that higher levels significantly increase risk. Fibrinogen (OR 1.8), BNP (OR 2.6), and Lp-PLA2 (OR 2.0) also exhibit significant, moderate associations. HbA1c (OR 1.16) shows a weaker but still statistically significant link. hs-TnI stands out with the highest OR of 3.4, indicating the strongest association with the outcome. All biomarkers have significant p-values ($p < 0.01$).

Table 8 illustrates the AUC, sensitivity, specificity, and p-value for various biomarkers, indicating their diagnostic performance. hs-CRP has an AUC of 0.78, with 75% sensitivity and 70% specificity, suggesting it is a moderately effective marker for identifying the condition. Fibrinogen has a lower AUC of 0.72 with 70% sensitivity and 65% specificity. HbA1c (AUC 0.75, sensitivity 74%, specificity 68%) and LDL-C (AUC 0.74, sensitivity 72%, specificity 66%) both show moderate diagnostic ability. hs-TnI has the highest AUC of 0.82, with 80% sensitivity and 73% specificity, indicating it is the most accurate biomarker. BNP (AUC 0.80, sensitivity 78%, specificity 70%) and Lp-PLA2 (AUC 0.77, sensitivity 73%, specificity 68%) also show significant diagnostic performance, all with p-values less than 0.001.

Figure 1 presents ROC curves for various biomarkers, including hs-CRP, Fibrinogen, HbA1c, LDL-C, hs-TnI, BNP, and Lp-PLA2. Each curve represents the trade-off between the true positive rate (sensitivity) and false positive rate (1 - specificity) for detecting a condition. The AUC values for each biomarker are shown, with hs-TnI having the highest AUC of 0.82, indicating the best diagnostic performance. A higher AUC implies better accuracy in distinguishing between the positive and negative classes. The dotted diagonal line represents random classification.

Table 8. Diagnostic accuracy ROC curve analysis.

Biomarker	AUC (95% CI)	Sensitivity	Specificity	p-value
hs-CRP	0.78	75%	70%	<0.001
Fibrinogen	0.72	70%	65%	<0.001
HbA1c	0.75	74%	68%	<0.001
LDL-C	0.74	72%	66%	<0.001
hs-TnI	0.82	80%	73%	<0.001
BNP	0.80	78%	70%	<0.001
Lp-PLA2	0.77	73%	68%	<0.001

hs-CRP: high-sensitivity C-Reactive Protein; HbA1c: Hemoglobin A1c; LDL-C: Low-Density Lipoprotein Cholesterol; hs-TnI: High-Sensitive Troponin I; BNP: B-type Natriuretic Peptide; Lp-PLA2: Lipoprotein-associated Phospholipase A2.

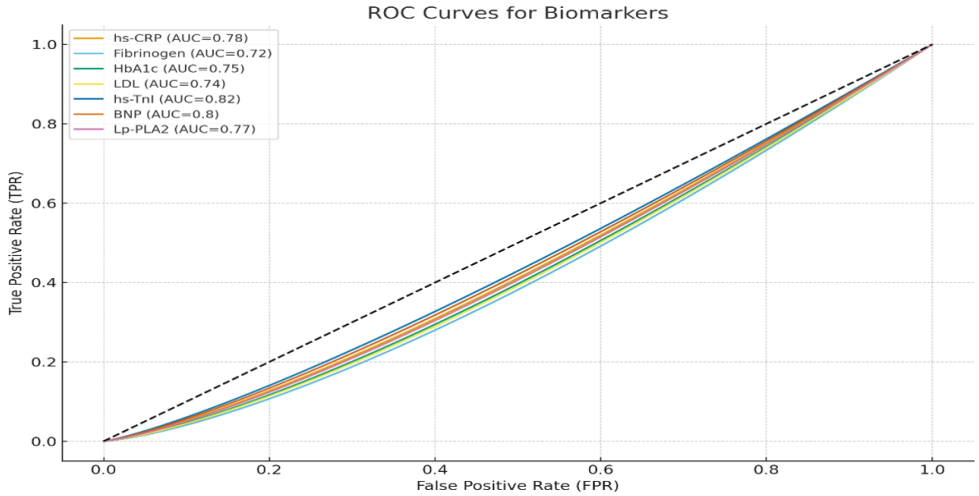

Figure 1. ROC curve for different biomarkers

Discussion

In this cross-sectional study of 1,200 adults with T2DM, we found that elevated inflammatory biomarkers (hs-CRP, fibrinogen), poor glycaemic control, and higher cardiac biomarker levels were strongly associated with established CVD and higher Framingham risk scores. hs-TnI, BNP, NT-proBNP, Lp-PLA2, and D-dimer showed strong associations with CVD presence and calculated risk categories, highlighting their potential value as part of multi-marker risk stratification in adults with T2DM.

Inflammation, Glycaemic Control, and Cardiovascular Risk

The progressive rise in hs-CRP across CVD risk categories in our cohort closely mirrors the findings of previous studies, which also demonstrated a strong positive correlation between hs-CRP and Framingham risk score in Indian T2DM patients.[7] Elevated hs-CRP has similarly been reported among Nigerian patients with diabetes, reinforcing inflammatory burden as a prominent predictor of cardiovascular risk in low-resource settings.[8]

Our observation shows that hs-CRP >3 mg/L was associated with 2.5-fold higher odds of prevalent CVD, aligning with other studies demonstrating that hs-CRP has been independently associated with vascular complications in longitudinal cohorts of diabetic individuals.[9] Additionally, results from the Rio de Janeiro T2DM cohort further support hs-CRP as a determinant of cardiovascular events.[10] The association between increasing HbA1c and higher calculated cardiovascular risk in our cross-sectional analysis is consistent with longitudinal studies and meta-analytic findings demonstrating that each 1% rise in HbA1c is linked to higher cardiovascular morbidity and mortality.[11] Improvements in glycaemic control have also been shown to parallel declines in hs-CRP.[12]

Cardiac Troponins and Myocardial Strain Markers

Our findings show that hs-TnI >10 ng/L was strongly associated with prevalent CVD (adjusted OR 3.6), aligning closely with large meta-analyses demonstrating that elevated hs-troponin levels independently predict major adverse cardiac events among diabetic patients.[13] The strong discriminative performance of hs-TnI (AUC 0.82) in distinguishing CVD status in our cross-sectional cohort aligns with evidence from longitudinal population-based studies showing that even low-grade troponin elevations have been associated with myocardial injury and are linked to future adverse events.[14]

BNP and NT-proBNP also showed strong associations with CVD and risk categories. Our results align with previous studies that reveal that NT-proBNP significantly improves prediction of coronary and renal outcomes in T2DM.[15] Additionally, other studies show that NT-proBNP identifies subclinical myocardial dysfunction in diabetics without overt heart failure, consistent with the elevated mean NT-proBNP in our cohort.[16]

Lp-PLA2, D-Dimer, and Vascular Risk

The association between Lp-PLA2 levels and prevalent CVD in our analysis is consistent with earlier longitudinal studies demonstrating that Lp-PLA2 is independently associated with coronary events in models that include traditional lipid measures.[17-18] Elevated Lp-PLA2 among diabetic individuals in our cohort is consistent with other findings, which demonstrated significantly higher levels among T2DM patients with ischemic stroke.[19]

Our results regarding D-dimer (OR 1.9 for >0.7 mg/L) are similar to other studies, which show that higher D-dimer levels predict future cardiovascular events in T2DM.[20] Furthermore, another study reported that elevated D-dimer is associated with poorer long-term outcomes among diabetics with acute coronary syndrome.[21]

Urban-Rural Variations

Rural participants exhibited significantly higher hs-CRP, HbA1c, troponin, BNP and D-dimer levels compared with urban participants. This pattern aligns with the other studies that reported greater inflammatory and metabolic derangements among rural or resource-limited diabetic populations.[8,22] These disparities are likely multifactorial and may reflect differences in health-care access, socioeconomic status and lifestyle patterns. These urban–rural comparisons are cross-sectional contrasts and do not demonstrate trajectories of risk or incidence of events over time.

Multimarker Approach and Clinical Implications

In multivariable logistic regression models, hs-CRP, fibrinogen, LDL-C, hs-TnI, BNP and Lp-PLA2 all remained independently associated with prevalent CVD. These findings support the potential utility of a multimarker strategy, as advocated in recent biomarker studies, indicating that combining markers of inflammation, myocardial injury, cardiac strain and vascular instability is associated with more detailed risk stratification beyond traditional factors alone; however, prospective studies are required to confirm prognostic utility and clinical benefit.[12,15-16]

Conclusion

In this large cross-sectional cohort of adults with T2DM in Punjab, systemic inflammation, subclinical myocardial injury, and vascular instability—reflected by elevated hs-CRP, fibrinogen, hs-troponins, BNP/NT-proBNP, D-dimer, and Lp-PLA2—were strongly associated with prevalent CVD and higher Framingham risk categories, independent of traditional risk factors. These biomarkers showed meaningful incremental associations with higher calculated risk strata beyond standard lipid and glycaemic indices and improved discrimination between participants with and without existing CVD. Rural participants displayed consistently higher biomarker levels and higher FRS values than urban participants, highlighting important inequities in cardiometabolic burden. Our findings support the potential integration of inflammatory and cardiac biomarkers into multimarker cardiovascular risk stratification frameworks for diabetes care, particularly in resource-limited settings. However, this is a cross-sectional analysis, temporal relationships cannot be established; therefore, prospective longitudinal studies are needed to establish true prognostic value and to determine whether biomarker-guided interventions can reduce future cardiovascular events in high-risk diabetic populations.

List of Abbreviations

ACE	Angiotensin-Converting Enzyme
ARBs	Angiotensin II Receptor Blockers
AUC	Area Under the Curve
BMI	Body Mass Index
BNP	B-type Natriuretic Peptide
BP	Blood Pressure
CIs	Confidence Intervals
CK-MB	Creatine Kinase-MB
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CMIA	Chemiluminescent Microparticle Immunoassay
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
eGFR	estimated Glomerular Filtration Rate
FRS	Framingham Risk Score
H-FABP	Heart-Type Fatty Acid-Binding Protein
HbA1c	Hemoglobin A1c
HDL-C	High-Density Lipoprotein Cholesterol
HPLC	High Performance Liquid Chromatography
hs-CRP	high-sensitivity C-Reactive Protein
hs-TnI	High-Sensitive Troponin I
hs-TnT	High-Sensitive Troponin T
JUPITER	Justification for the Use of Statins in Prevention
LDL-C	Low-Density Lipoprotein Cholesterol
Lp-PLA2	Lipoprotein-associated Phospholipase A2
NGSP	National Glycohemoglobin Standardization Program
NT-proBNP	N-Terminal pro-B-type Natriuretic Peptide
OR	Odds Ratio
ROC	Receiver Operating Characteristic
SBP	Systolic Blood Pressure
T2DM	type 2 diabetes
TC	Total Cholesterol
TG	Triglycerides

Ethical Clearance

The study was approved by the Institutional Ethics Committee of Rayat Bahra Professional University, and written informed consent was obtained from all participants before their inclusion in the study. All procedures were conducted in compliance with the Declaration of Helsinki and ethical guidelines for human research

Publication Approval

All authors consent to the publication of this manuscript.

Authors' Contributions

All authors have contributed equally to the research.

Acknowledgments

None.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Availability of Data and Materials

Not applicable.

Funding

This paper received no specific grant from any funding agency, commercial or not-for-profit sectors."

Copyright/Permissions for Figures

Not applicable.

Generative AI and AI-Assisted Technologies in the Writing Process

We hereby declare that Generative AI and AI-assisted technologies were used only for language-related assistance during the preparation of the manuscript, including improving grammar, sentence structure, clarity, readibility, and overall English language presentation. Such tools were not used to generate, fabricate, manipulate, or intepret the study data, statistical results, scientific findings, or references.

The authors retain full responsibility for the accuracy, originality, integritym and scientific content of the manuscript. All scientific concepts, study design, methodologym data interpretation, conclusions, and final content have been reviewed and approved by the authors. The authors have independently verified the information presented in the manuscript and accept full responsibility for its content.

We confirm that the use of AI-assissted technologies does not alter the authorship, accountability, or originality of the work, and that the manuscript complies with the journal's policies regarding the responsible use and disclosure of AI technologies.

References

1. International Diabetes Federation. (2023). IDF Diabetes Atlas (10th ed.). Brussels, Belgium: International Diabetes Federation.
2. American Diabetes Association. (2020). Classification and diagnosis of diabetes: Standards of medical care in diabetes-2020. Diabetes Care, 43(Suppl 1), S14-S31. https://doi.org/10.2337/dc20-S002
3. Ridker, P. M., Rifai, N., Rose, L., et al. (2002). Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. New England Journal of Medicine, 347(20), 1557-65.
4. Pradhan, A. D., Manson, J. E., Rifai, N., et al. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA, 286(3), 327-34.
5. Ridker, P. M., Danielson, E., Fonseca, F. A., et al. (2008). Rosuvastatin to prevent vascular events in men and women with elevated CRP. New England Journal of Medicine, 359(21), 2195-207.
6. Anjana, R. M., Deepa, M., Pradeepa, R., et al. (2017). Prevalence of diabetes and prediabetes in 15 states of India. Lancet Diabetes & Endocrinology, 5(8), 585-96.
7. Mahadevaiah, D. (2025). Association between high-sensitivity C-reactive protein and cardiovascular risk in type 2 diabetics. European Journal of Cardiovascular Medicine, 15(6), 301-304.
8. Mohammed, J. A., Basil, B., Mba, I. N., et al. (2025). Elevated hs-CRP and dyslipidaemia in type 2 diabetes mellitus: Implications for cardiovascular risk. BMC Endocrine Disorders, 25(1), 100.
9. Pfützner, A., & Forst, T. (2006). High-sensitivity CRP as a cardiovascular risk marker in diabetes mellitus. Diabetes Technology & Therapeutics, 8(1), 28-36.
10. Cardoso, C. R. L., Leite, N. C., & Salles, G. F. (2016). Prognostic importance of CRP in high-risk T2DM: Rio de Janeiro Cohort. Journal of the American Heart Association, 5(11), e004554.
11. Cavero-Redondo, I., Peleteiro, B., Alvarez-Bueno, C., et al. (2017). HbA1c and cardiovascular outcomes: Systematic review and meta-analysis. BMJ Open, 7, e015949.
12. Song, T., Lan, Y., Li, K., et al. (2023). Prognostic value of high-sensitivity cardiac troponin in diabetes: Meta-analysis. PeerJ, 11, e16376.
13. Petchiappan, V., Sivakrishna, N., Manickam, S., & Menon, S. (2019). Glycaemic control and CRP levels in T2DM: A prospective study. International Journal of Research in Medical Sciences, 7(5), 1818-1821.
14. Ridker, P. M., Buring, J. E., & Cook, N. R. (2003). CRP, metabolic syndrome and vascular risk in healthy women. Circulation, 107, 391-397.
15. Ma, R. C. W., et al. (2025). NT-proBNP improves prediction of cardiorenal disease in type 2 diabetes. Diabetologia. Advance online publication.
16. Gastouniotis, I., et al. (2024). Clinical

significance of NT-proBNP in T2DM without known CVD. Biomedicines, 12(12), 2718.
17. Koenig, W., Twardella, D., Brenner, H., & Rothenbacher, D. (2006). Lp-PLA2 predicts future cardiovascular events. Arteriosclerosis, Thrombosis, and Vascular Biology, 26(7), 1586-1593.
18. Maiolino, G., Pedon, L., Cesari, M., et al. (2012). Lp-PLA2 activity and cardiovascular events in high-risk patients. PLoS One, 7(10), e48171.
19. Shen, H., et al. (2025). Clinical value of Lp-PLA2 and inflammatory markers in T2DM with stroke. Frontiers in Endocrinology, 16, 1546961.
20. Cheng, L., Fu, Y., Cao, X., et al. (2022). D-dimer predicts cardiovascular outcomes in type 2 diabetes. Cardiovascular Diabetology, 21, 39.
21. Zhou, B. Y., et al. (2022). D-dimer and long-term prognosis in T2DM with ACS. Nutrition, Metabolism & Cardiovascular Diseases, 32, 2705-15.
22. Khattab, M. H., Shahwan, M. J., Hassan, N. A. G. M., et al. (2022). hs-CRP and cardiovascular-renal risk in T2DM. Rev Diabet Stud, 18(1), 27-33.