Integrated Assessment of Endothelial Dysfunction, Inflammation and Cardiometabolic Risk in Coronary Artery Disease with and without Type 2 Diabetes Mellitus

Running Title: *Integrated Endothelial and Cardiometabolic Biomarkers in CAD and Type 2 Diabetes*

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Background:

Coronary artery disease (CAD) and type 2 diabetes mellitus (T2DM) are closely associated with endothelial dysfunction, chronic inflammation, hyperglycaemia, and dyslipidaemia, all of which contribute to accelerated atherosclerosis. However, studies with integrating biomarkers of endothelial dysfunction, systemic inflammation, glycaemic status, and cardiometabolic risk in patients with CAD with and without T2DM remain limited. The present study aimed to evaluate serum nitrite, high-sensitivity C-reactive protein (hs-CRP), fasting plasma glucose (FPG), and lipid profile as interrelated biomarkers of endothelial dysfunction, inflammation, and cardiometabolic risk.

Methods:

This hospital-based cross-sectional comparative study included 300 participants (30–75 years), equally allocated into four groups: healthy controls (n = 75), T2DM (n = 75), CAD (n = 75), and CAD with T2DM (n = 75). Serum nitrite (NO_2^-) was quantified by high-performance liquid chromatography (HPLC) as an indirect marker of nitric oxide bioavailability. Fasting plasma glucose was estimated by the glucose oxidase–peroxidase method, hs-CRP by high-sensitivity immunoturbidimetry, and lipid profile by standard enzymatic methods. Data were analysed using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered statistically significant.

Results:

Age was comparable among the study groups ($F = 2.099$, $p = 0.100$). Fasting plasma glucose increased significantly from healthy controls (82.9 ± 8.21 mg/dL) to T2DM (188.23 ± 25.6 mg/dL), CAD (90.12 ± 10.3 mg/dL), and CAD with T2DM (269.4 ± 25.8 mg/dL) ($F = 1577.193$, $p < 0.001$). Mean hs-CRP increased progressively from 0.85 ± 0.42 mg/L to 4.60 ± 1.52 mg/L, 7.09 ± 2.61 mg/L, and 11.01 ± 1.30 mg/L, respectively ($F = 497.958$, $p < 0.001$). Serum nitrite showed a significant decline from 34.46 ± 6.32 µmol/L in healthy controls to 28.12 ± 7.49 µmol/L in T2DM, 17.12 ± 4.36 µmol/L in CAD, and 12.46 ± 2.13 µmol/L in CAD with T2DM ($F = 253.540$, $p < 0.001$). Total cholesterol, triglycerides, LDL-C, VLDL-C, and non-HDL-C increased significantly, whereas HDL-C decreased progressively across the study groups (all $p < 0.01$), with the most pronounced abnormalities observed in CAD with T2DM patients.

Conclusion:

Patients with CAD with T2DM exhibited the greatest impairment in glycaemic control, systemic inflammation, endothelial function, and lipid metabolism. The combined assessment of fasting plasma glucose, hs-CRP, serum nitrite, and lipid profile provides a comprehensive evaluation of cardiometabolic and endothelial dysfunction and may improve cardiovascular risk stratification beyond individual biomarkers. Serum nitrite appears to be a promising adjunct biomarker for assessing endothelial dysfunction in patients with CAD and T2DM. Further multicentre prospective studies are warranted to validate its prognostic utility.

Keywords:

Coronary artery disease; Type 2 diabetes mellitus; Endothelial dysfunction; Serum nitrite; Nitric oxide; High-sensitivity C-reactive protein; Dyslipidaemia; Cardiometabolic risk.

How to cite this article: Badade ZG, Mahendra SY, Sandeep R, Shilpa K, Datta B, Sagar S. Integrated Assessment of Endothelial Dysfunction, Inflammation and Cardiometabolic Risk in Coronary Artery Disease with and without Type 2 Diabetes Mellitus. Int J Drug Deliv Technol. 2026;16(70s): 529-536. DOI: 10.25258/ijddt.16.70s.53

Introduction:

Coronary artery disease (CAD) continues to be the leading cause of death and disability worldwide, despite major advances in preventive cardiology and interventional therapies. The pathogenesis of CAD is multifactorial, involving endothelial dysfunction, chronic inflammation, dyslipidemia, hyperglycemia, oxidative stress and traditional cardiovascular risk factors. Endothelial dysfunction is increasingly recognized as one of the earliest detectable abnormalities preceding atherosclerosis and as an important contributor to the initiation and progression of coronary artery disease (1). The vascular endothelium is a metabolically active organ that regulates vascular homeostasis through control of vascular tone, inhibition of platelet aggregation, suppression of leukocyte adhesion, and modulation of inflammatory responses. The major endothelium-derived vasodilator is nitric oxide (NO), which is synthesized from L-arginine by endothelial nitric oxide synthase (eNOS). Besides vasodilation, NO has anti-inflammatory, anti-thrombotic, antioxidant and antiproliferative properties protecting the vascular wall. Oxidative stress, endothelial injury and impaired eNOS activity decrease nitric oxide bioavailability, which is now considered a hallmark of endothelial dysfunction and contributes significantly to the pathogenesis of atherosclerosis and coronary artery disease (1,2). The role of inflammation as a key player in the pathogenesis of atherosclerosis and its complications is increasingly appreciated. High-sensitivity C-reactive protein (hs-CRP), an acute-phase reactant derived from the liver, is induced by inflammatory cytokines and has emerged as an important biomarker of residual inflammatory cardiovascular risk. Elevated hs-CRP concentrations have been associated with endothelial dysfunction, decreased nitric oxide bioavailability, plaque instability, and adverse cardiovascular outcomes, irrespective of conventional lipid abnormalities. Persistent vascular inflammation leads to worsening endothelial dysfunction and creates a vicious cycle that accelerates coronary artery disease (1,3). Cardiometabolic abnormalities such as hyperglycemia, dyslipidemia, etc., further aggravate endothelial dysfunction and inflammatory activation. Chronic hyperglycemia causes the formation of advanced glycation end products, oxidative stress and reduced nitric oxide production, and high low-density lipoprotein cholesterol and triglycerides promote lipid deposition in the arterial wall and increase the progression of atherosclerotic plaque. These metabolic derangements synergize with inflammation and endothelial dysfunction to enhance cardiovascular morbidity and mortality (2). Although nitric oxide, hs-CRP, fasting blood glucose and lipid parameters have been studied individually in patients with coronary artery disease, these biomarkers are encompassed in distinct but interrelated pathophysiological domains, namely endothelial dysfunction, systemic inflammation, glycemic dysregulation and cardiometabolic risk. (1,3). To our knowledge, few studies have simultaneously assessed endothelial dysfunction, systemic inflammation, glycemic status and lipid abnormalities in Indian patients with CAD with and without T2DM. The present study was undertaken to perform an integrated cardiometabolic and endothelial biomarker profiling in patients with coronary artery disease by evaluating the nitric oxide bioavailability, high-sensitivity C-reactive protein, fasting blood glucose, lipid profile and demographic characteristics. Such integrated approaches may improve the understanding of the interplay between endothelial dysfunction, inflammation and metabolic abnormalities in CAD and may provide valuable insights for comprehensive cardiovascular risk assessment.

Materials and Methods:

Study Design and Setting; This hospital based cross sectional comparative study was done in the Department of Biochemistry in collaboration with the Departments of Cardiovascular Thoracic Surgery, General Medicine and Cardiology. Ethical approval was obtained from Institutional Ethics Committee. Written informed consent was obtained from all the participants.

Study Population and sample size:

Sample size was calculated according to previously published biomarker studies evaluating inflammatory and endothelial dysfunction markers in patients with Type 2 Diabetes Mellitus and Coronary Artery Disease, to ensure adequate statistical power for intergroup comparisons.

A total of 300 participants (30 – 75 years) were enrolled and equally allocated (n=75) in four groups as include for all four groups:

Group I: Healthy controls (30-75 years)

Patient group II: Patients with T2DM diagnosed according to ADA 2024 criteria without clinical manifestation of CAD

Group III: Patients with angiographically proven CAD (≥50% stenosis) without diabetes

Group IV: T2DM patients with angiographically proven CAD

Exclusion Criteria:

Type 1 diabetes mellitus Chronic kidney disease Chronic liver disease Autoimmune or inflammatory diseases, Malignancy, Pregnancy, Acute Infection, and Recent surgery or trauma.

Sample Collection: Venous blood (5-8 mL) was collected after an overnight fasting of 10–12 h. The serum was separated by centrifugation at 3000 rpm for 10 min and stored at −20°C until analysis.

Biochemical Analysis: Serum nitrite (NO_2^-), a stable oxidation product of nitric oxide, was measured by HPLC according to the method of Wu et al. (2013) as an indirect surrogate marker of nitric oxide bioavailability and endothelial function. High-sensitivity C-reactive protein (hs-CRP): Determined by high-sensitivity immunoturbidimetric assay. Fasting Blood Sugar (FBS): Estimated by the glucose oxidase – peroxidase (GOD – POD) method. Lipid Profile: Total cholesterol, triglycerides, HDL-C enzymatically measured. LDL-C and VLDL-C calculated by Friedewald equation if applicable.

Statistical analysis was performed with SPSS Version 25.0. Normality of continuous variables was assessed using Shapiro–Wilk test and homogeneity of variances was evaluated using Levene's test. Continuous variables were presented as mean ± standard deviation (SD). We compared the four study groups using one-way analysis of variance (ANOVA) with multiple pairwise comparisons using Tukey's post hoc test. Statistical significance was defined as a two-sided p-value <0.05.

Results:

Table No 1: Comparison of Age, Fasting Plasma Glucose, hs-CRP and Serum Nitrite Concentration (Indirect Marker of Nitric Oxide Bioavailability) Between Healthy Controls, T2DM, CAD and CAD with T2DM Patients

Parameter	Healthy Controls (n=75)	T2DM (n=75)	CAD (n=75)	CAD with T2DM (n=75)	F-value	P-value
Age	53.75 ± 6.35	54.75 ± 6.12a	54.15 ± 4.23b, d	55.75 ± 3.53c, e, f	2.099	0.100 NS
Fasting Plasma Glucose (mg/dL)	82.9 ±8.21	188.23±25.6a*	90.12 ±10.3b, d*	269.4 ±25.8c*e, f**	1577.193	< 0.001
hs-CRP (mg/L)	0.85 ±0.42	4.6±1.52a*	7.09± 2.61b*, d*	11.01 ±1.3c*, e**, f*	497.958	< 0.001
Serum Nitrite (NO_2^-) (µmol/L)	34.46±6.32	28.12 ±7.49a*	17.12 ±4.36b**, d**	12.46 ±2.13c*, e**, f*	253.540	< 0.001

Values are expressed as Mean ±SD, p*≤0.05 significant, p**≤0.01 highly significant with 95 % CI and a, b, c, d, e, & f without asteric shows non-significant difference. Table no. 1 shows a) Age, Fasting Plasma Glucose, hs-CRP and Serum Nitrite Concentration comparison in control to T2DM, b) Comparison of control to CAD, c) Comparison of control to T2DM with CAD, d) Comparison of T2DM to CAD, e) Comparison of T2DM to CAD with T2DM and f) Comparison of CAD to CAD with T2DM.

Table No.2: Comparison of Lipid Profile Among Healthy Controls, T2DM, CAD and CAD with T2DM

Values are expressed as Mean ±SD, p*≤0.05 significant, p**≤0.01 highly significant with 95 % CI and a, b, c, d, e, & f without asteric shows non-significant difference. Table no. 2 shows a) Comparison of lipid profile in control to T2DM, b) Comparison of control to CAD, c) Comparison of control to T2DM with CAD, d) Comparison of T2DM to CAD, e) Comparison of T2DM to CAD with T2DM and f) Comparison of CAD to CAD with T2DM.

Parameters	Control	T2DM	CAD	CAD + T2DM	F value	P value
T. Cholesterol (mg/dl)	147.7±15.6	212.1±17.1a*	223.6±10.1b*, d*	238.2±15.4c***, e* f*	546.47	<0.01 S
TG (mg/dl)	98.4±22.2	164.5±18.3 a*	188.2±20.9b**, d	201.6±11.7 c**, e*, f	449.56	<0.01 S
HDL-C (mg/dl)	56.8±4.85	48.01±5.6a*	38.5±2.5b*, d*	37.6±1.6 c**, e*, f	384.82	<0.01 S
LDL-C (mg/dl)	71.22±10.21	131.19±16.7a*	147.46±15.2b*, d*	160.28±13.5c*, e**, f*	123.542	<0.01 S
VLDL-C (mg/dl)	19.68±3.5	32.90±4.6a*	37.64±6.5b*, d	40.32±3.8c*, e*, f	449.56	<0.01 S
Non-HDL-C (mg/dl)	90.90±36.2	164.09±28.4a*	185.10±32.5b*, d*	200.60±31.6c*, e*, f	753.20	<0.01 S

Discussion:

The present study consisted of 300 subjects equally divided into four groups as healthy controls, T2DM, CAD and CAD with T2DM, 75 subjects in each group with the mean age was equivalent across all groups. This permitted a balanced assessment of metabolic, inflammatory and endothelial indicators. Endothelial dysfunction, oxidative stress, vascular stiffness and persistent low-grade inflammation are related with advanced age. The 2023 ESC Guidelines further underline that diabetes is a key driver of cardiovascular risk and that thorough risk assessment and management are needed in individuals with diabetes and established cardiovascular disease (4). Therefore, the comparable age distribution in the present investigation supports the view that the observed variations in fasting plasma glucose, hs-CRP and serum nitrite are predominantly disease-related rather than age-related. In the present investigation, fasting plasma glucose demonstrated a very highly significant difference among the four groups ($F=1577.193$, $p<0.001$). The highest fasting plasma glucose was recorded in CAD with T2DM patients (269.4 ± 25.8 mg/dL) followed by T2DM patients (188.23 ± 25.6 mg/dL) whereas healthy controls and CAD patients without diabetes showed comparably lower levels. This discovery is biologically feasible as chronic hyperglycaemia is the key biochemical aberration in T2DM. However, the noted elevated glucose level in CAD with T2DM patients supports a greater metabolic burden in patients with concurrent diabetes and cardiovascular disease. Our result is consistent with Marx et al. who said in the 2023 ESC Guidelines that individuals with T2DM and established atherosclerotic cardiovascular disease are a high-risk category that needs rigorous care of hyperglycemia and cardiovascular risk factors. The ADA Standards of Care also indicate that cardiovascular disease is the primary cause of morbidity and mortality in diabetes and glycaemic control should be integrated with cardiovascular risk management (4). Hyperglycaemia causes endothelial damage by several pathways such as increased production of reactive oxygen species, activation of protein kinase C, creation of advanced glycation end products (AGEs), mitochondrial dysfunction, and reduced nitric oxide bioavailability. Adverse endothelial function and coronary atherosclerosis have also been linked to poor glycaemic management. In T2DM patients, inadequate glycaemic management has been shown to increase endothelial dysfunction and aggravate coronary atherosclerosis (Chen et al 5). This is consistent with our result that the CAD with T2DM group had the highest fasting plasma glucose and the lowest serum nitrite content. The interplay of hyperglycaemia and endothelial dysfunction may thus explain the increased susceptibility of diabetic patients to accelerated coronary artery disease. Uncontrolled hyperglycaemia persists and induces an increase in oxidative stress and generation of advanced glycation end products (AGEs). These AGEs activate the AGE–RAGE/NF-κB signalling pathway, resulting in increased production of pro-inflammatory cytokines (IL-6 and TNF-α) and a subsequent rise in hs-CRP. At the same time, oxidative stress induces decreased endothelial nitric oxide synthase (eNOS) activity and nitric oxide bioavailability, which cause endothelial dysfunction and accelerated atherosclerosis (1, 6-8).

High-sensitivity C-reactive protein was gradually and significantly higher from healthy controls to T2DM, CAD and CAD with T2DM patients ($F = 497.958$, $p < 0.001$). The mean hs-CRP level was the least in healthy controls (0.85 ± 0.42 mg/L) and increased in T2DM patients (4.60 ± 1.52 mg/L), further increased in CAD patients (7.09 ± 2.61 mg/L) and was greatest in CAD with T2DM patients (11.01 ± 1.30 mg/L). This gradual increment suggests a progressive systemic inflammatory burden as the disease evolves from metabolic disease to coronary disease and finally to combination diabetic coronary pathology. Our results are consistent with Liu et al. who found a linear positive correlation of hs-CRP with prevalence of cardiovascular disease among patients with diabetes. In their analysis, higher hs-CRP quartiles were substantially linked with increased prevalence of CVD after adjusting for covariates (9). This validates the present observation that hs-CRP was significantly higher in CAD patients with T2DM. Moreover, recent reviews have highlighted that hs-CRP is a marker of residual inflammatory risk in atherosclerotic cardiovascular disease and can be used to identify patients with ongoing vascular inflammation (10). The elevation of hs-CRP in diabetic and CAD patients may be due to continuous activation of inflammatory pathways. Hyperglycaemia, insulin resistance, obesity, dyslipidaemia and oxidative stress promote pro-inflammatory cytokines, especially interleukin-6 and tumour necrosis factor-alpha. These cytokines stimulate the liver to produce CRP. hs-CRP in CAD also indicates vascular inflammation, plaque instability, endothelial activation and macrophage infiltration within atherosclerotic plaques. Thus, the greatest hs-CRP level in CAD with T2DM patients shows the combined inflammatory effect of diabetes and coronary atherosclerosis.

The present investigation demonstrates that increased systemic inflammation is related to decreased nitric oxide availability. Higher hs-CRP reduces the production of endothelial nitric oxide synthase (eNOS) and raises oxidative stress, which causes more degradation of nitric oxide and endothelial dysfunction. Therefore, decreased nitric oxide availability contributes to poor vasodilation, vascular inflammation, and development of atherosclerosis (1,11).

There was a significant gradual drop of serum nitrite content, an indirect index of the bioavailability of nitric oxide, and endothelial function among groups ($F = 253.540$, $p < 0.001$). The present investigation indicated that serum nitrite content gradually decreases with increasing severity of the condition. The serum nitrite levels were highest in healthy controls (34.46 ± 6.32 µmol/L), followed by a significant reduction in T2DM patients (28.12 ± 7.49 µmol/L), a further decline in CAD patients (17.12 ± 4.36 µmol/L) and the lowest concentration in patients with CAD associated with T2DM (12.46 ± 2.13 µmol/L), suggesting progressive deterioration of endothelial nitric oxide bioavailability.

Bahadoran et al. significantly confirm this conclusion describing vascular nitric oxide resistance as a typical hallmark of T2DM (8). Impaired nitric oxide signaling is also associated with cardiovascular events and death in diabetes individuals (8). Nitric oxide is an important endothelial mediator that mediates vasodilation, inhibits platelet aggregation, suppresses proliferation of vascular smooth muscle and prevents leukocyte adherence. Reduced bioavailability of nitric oxide leads to vasoconstriction, thrombosis, inflammation and development of atherosclerosis. Moreover, Ikonomidis et al. observed that endothelial dysfunction is related with lower nitric oxide bioavailability in patients with inadequate glycaemic control and first-degree relatives of patients with T2DM (13). Their findings are consistent with the present observation where blood nitrite levels were decreased already in T2DM patients as compared to controls and further decreased in CAD and CAD with T2DM patients. This shows that nitric oxide dysfunction may start early in diabetes and progress as coronary artery disease develops.

The cause for decreased serum nitrite in T2DM and CAD is complex. Hyperglycaemia increases superoxide production, which rapidly interacts with nitric oxide to create peroxynitrite, hence lowering nitric oxide bioavailability. Oxidative stress also uncouples endothelial nitric oxide synthase and causes the enzyme to produce superoxide instead of nitric oxide. In addition, advanced glycation end products inhibit function of endothelial nitric oxide synthase and induce endothelial inflammation (14). These processes are in agreement with the lowest concentration of nitrite in patients with CAD and T2DM in the present investigation.

The joint pattern of raised fasting plasma glucose, elevated hs-CRP and lower serum nitrite in CAD with T2DM patients shows a close association between hyperglycaemia, inflammation and endothelial dysfunction. Chronic hyperglycaemia may induce oxidative damage and inflammatory activation. Inflammation also affects the endothelium and promotes the development of atherosclerotic plaques. Endothelial dysfunction is associated with decreased nitric oxide availability leading to poor vasodilation, platelet activation, vascular stiffness and coronary plaque instability.

Recently, Wang et al. concluded that endothelial dysfunction is characterized by decreased nitric oxide availability, oxidative stress, inflammatory activation and vascular injury (15). This is

consistent with the biological explanation of the present findings. Similarly, Medina-Leyte et al. addressed the important role of endothelial dysfunction and inflammation in the etiology of coronary artery disease (1). Thus, the present investigation provides biochemical evidence that CAD patients with T2DM have the most severe combined metabolic, inflammatory and endothelial dysfunction. The results indicate that early detection of high levels of hs-CRP and low levels of nitrite may help to identify those diabetic individuals at high risk who need intensive cardiovascular preventive treatments.

These findings together indicate that hyperglycemia, inflammation and endothelial dysfunction coexist and may synergistically contribute to the development of coronary artery disease. Because dyslipidemia is the key mechanism in the acceleration of atherosclerosis, the lipid profile was further assessed to measure the degree of cardiometabolic derangements across the study groups.

The present study demonstrated a progressive deterioration of the lipid profile from healthy controls to T2DM, CAD and CAD with T2DM patients, suggesting that diabetes and coronary artery disease synergistically worsen atherogenic dyslipidemia. Total cholesterol, triglycerides, LDL-C, VLDL-C and non-HDL-C significantly increased and HDL-C decreased progressively across the study groups. Total cholesterol was increased from 147.7 ± 15.6 mg/dL in healthy controls to 212.1 ± 17.1 mg/dL in T2DM, 223.6 ± 10.1 mg/dL in CAD and 238.2 ± 15.4 mg/dL in CAD with T2DM patients ($F = 546.47$, $P < 0.01$). Likewise, triglycerides were increased from 98.4 ± 22.2 mg/dL to 164.5 ± 18.3 mg/dL, 188.2 ± 20.9 mg/dL and 201.6 ± 11.7 mg/dL respectively ($F = 449.56$, $P < 0.01$). Progressive significant increases were also observed in LDL-C, VLDL-C and non-HDL-C with a decrease in HDL-C from 56.8 ± 4.85 mg/dL in controls to 37.6 ± 1.6 mg/dL in patients with CAD and T2DM ($F = 384.82$, $P < 0.01$). Together these findings suggest an increase in cardiometabolic derangement with increasing disease severity. Our results agree with Wang et al. who found significantly higher total cholesterol, triglycerides and LDL-C and lower HDL-C in patients with type 2 diabetes compared to healthy controls. They showed that the deterioration of glycemic control was closely associated with progressive deterioration of lipid metabolism, supporting the concept that hyperglycemia and dyslipidemia coexist because of insulin resistance and altered hepatic lipid metabolism (16). Similarly, Addis Z et al described diabetic dyslipidemia as the characteristic metabolic abnormality consisting of elevated triglycerides, increased VLDL production, increased small dense LDL particles and reduced HDL-C secondary to impaired insulin action (17).

These findings are also consistent with Banach et al. who pointed out that high LDL-C remains the main causal factor for atherosclerotic cardiovascular disease and that combined dyslipidemia substantially amplifies residual cardiovascular risk despite conventional therapy (18). Likewise, the ESC/EAS Guidelines for the management of dyslipidaemias recommend intensive LDL-C lowering in patients with established coronary artery disease and diabetes, since these patients are classified as being at very high cardiovascular risk (19). The present study strongly supports these recommendations as the levels of LDL-C (160.28 ± 13.5 mg/dL) and non-HDL-C (200.60 ± 31.6 mg/dL) were significantly higher in the CAD with T2DM group. Furthermore, our findings are consistent with those of Lan et al. showing a close temporal association between atherogenic dyslipidemia and systemic inflammation. Their longitudinal cohort study showed that high levels of triglycerides, non-HDL cholesterol and low HDL-C were associated with persistent low-grade inflammation and increased cardiometabolic risk, and suggested that dyslipidemia itself promotes chronic inflammatory activation (20). This finding is particularly pertinent to the present study in which CAD patients with T2DM showed the worst lipid profile and the highest hs-CRP levels, indicating the possible synergistic effect of dyslipidemia and inflammation on atherogenesis.

The metabolic implications of insulin resistance can account for the progressive dyslipidemia observed in this study. Decreased insulin activity increases the activity of hormone-sensitive lipase in adipose tissue and leads to an increased release of free fatty acids into the circulation. Excess free fatty acids are transported to the liver where they promote hepatic triglyceride synthesis and overproduction of VLDL particles. The subsequent exchange mediated by cholesteryl ester transfer protein (CETP) results in enrichment of LDL and HDL particles with triglycerides, leading to small dense LDL particles and accelerated HDL catabolism. Small dense LDL particles are more prone to oxidative modification, have an increased ability to cross the vascular endothelium and are rapidly taken up by macrophages resulting in foam cell formation and progression of atherosclerotic plaques. Moreover, low HDL-C impairs reverse cholesterol transport, antioxidant activity and endothelial protection, further aggravating vascular injury and inflammation (16–18).

The significantly reduced HDL-C together with markedly elevated levels of total cholesterol, triglycerides, LDL-C, VLDL-C and non-HDL-C in

CAD with T2DM patients indicate the presence of advanced atherogenic diabetic dyslipidemia. These lipid abnormalities together with hyperglycemia, chronic inflammation and endothelial dysfunction create a pro-atherogenic milieu that accelerates the process of coronary plaque formation, vascular remodelling and adverse cardiovascular outcomes. Thus, the assessment of the lipid profile in combination with inflammatory and endothelial markers may be more informative than isolated biochemical parameters in the evaluation of cardiovascular risk. Thus, persistent hyperglycaemia promotes dyslipidaemia, oxidative stress, and chronic low-grade inflammation, leading to endothelial dysfunction and reduced nitric oxide bioavailability.

Conclusion:

In the present study, we found a progressive increase in FPG and hs-CRP with a significant reduction in serum nitrite concentration in healthy controls, T2DM, CAD and CAD with T2DM patients. CAD with T2DM group showed the most significant biochemical changes, indicating the synergistic effects of metabolic dysregulation, systemic inflammation and endothelial dysfunction. These findings suggest that an evaluation of cardiometabolic risk incorporating glycemic status, inflammatory load, endothelial dysfunction, and lipid abnormalities is more complete than any single biomarker. Serum nitrite, an indirect marker of nitric oxide bioavailability, may be a useful adjunct biomarker for early cardiovascular risk stratification in patients with T2DM and CAD. Further multi-centre prospective studies are required to validate our findings and to assess their prognostic significance in predicting adverse cardiovascular outcomes.

Limitations:

Limitations of the present study include the single-centre and cross-sectional design and the use of serum nitrite as an indirect marker of nitric oxide bioavailability. Further multicentre prospective studies are needed to confirm these findings.

Acknowledgements:

Authors are highly thankful to Department of Biochemistry, MGM Medical College and Hospital, Kamothe, Navi Mumbai for providing the facilities and support to carry out this study. The authors also thank the Departments of General Medicine and Cardiology, the study subjects, the technical staff and the Institutional Ethics Committee for their valuable cooperation and support.

Funding: This project is self-funded.

Conflict of Interest: The authors declare that they have no conflict of interest.

References:

1. Medina-Leyte DJ, Zepeda-García O, Domínguez-Pérez M, González-Garrido A, Villarreal-Molina T, Jacobo-Albavera L. Endothelial dysfunction, inflammation and coronary artery disease: potential biomarkers and promising therapeutical approaches. Int J Mol Sci. 2021;22(8):3850.
2. Roy R, Marín-Palma D, Banerjee J, et al. Dysfunctional and dysregulated nitric oxide synthases in cardiovascular disease. Int J Mol Sci. 2023;24(20):15200.
3. Maio R, Sciatti E, et al. Endothelial dysfunction and C-reactive protein predict cardiovascular outcomes. ESC Heart Fail. 2021; 8:457–466.
4. Marx N, Federici M, Schütt K, Müller-Wieland D, Ajjan RA, Antunes MJ, et al. 2023 ESC Guidelines for the management of cardiovascular disease in patients with diabetes. Eur Heart J. 2023;44(39):4043-4140.
5. Chen S, Guo L, Zhang Y, et al. Impact of glycemic control on the association of endothelial dysfunction and coronary atherosclerosis in patients with type 2 diabetes mellitus. Cardiovasc Diabetol. 2021; 20:64.
6. Taguchi K, Fukami K. RAGE signaling regulates the progression of diabetic complications. *Front Pharmacol*. 2023; 14:1128872.
7. González P, Lozano P, Ros G, Solano F. Hyperglycemia and oxidative stress: An integral, updated and critical overview of their metabolic interconnections. *Int J Mol Sci.* 2023;24(11):9352.
8. Bahadoran Z, Carlström M, Mirmiran P, Kashfi K, Ghasemi A. Vascular nitric oxide resistance in type 2 diabetes. *Cell Death Dis*. 2023; 14:410.
9. Liu Y, He W, et al. A linear positive association between high-sensitivity C-reactive protein and the prevalence of cardiovascular disease among individuals with diabetes. BMC Cardiovasc Disord. 2024; 24:435.
10. Mehta A, Blumenthal RS, Gluckman TJ, Feldman DI, Kohli P. High-sensitivity C-reactive protein in atherosclerotic cardiovascular disease: To measure or not to measure? *US Cardiol.* 2025;19: e06.
11. Mohammadnia N, Banach M, Penson PE, Sahebkar A. How to effectively treat residual risk in atherosclerotic cardiovascular disease. *Heart Lung Circ.* 2023;32(11):1465-1478.
12. Dri E, Romanello M, et al. Inflammatory mediators of endothelial dysfunction. *Life (Basel)*. 2023;13(6):1420.

13. Ikonomidis I, et al. Endothelial dysfunction is associated with decreased nitric oxide bioavailability in dysglycaemic subjects and first-degree relatives of type 2 diabetic patients. J Clin Med. 2022; 11:3579.
14. An Y, Zhang X, Wang Y, et al. The role of oxidative stress in diabetes mellitus-induced endothelial dysfunction. Signal Transduction and Targeted Therapy. 2023; 8:223.
15. Wang X, et al. Endothelial dysfunction: molecular mechanisms and clinical implications. Signal Transduct Target Ther. 2024; 9:156.
16. Wang L, Zhang Y, Liu X, et al. The association between blood glucose levels and lipids or lipid ratios in type 2 diabetes. Front Endocrinol (Lausanne). 2022; 13:969080.
17. Addis Z, Ayele Y, Belete Y, et al. Dyslipidemia and associated factors among adult patients with type 2 diabetes mellitus: a cross-sectional study. **Front Cardiovasc Med.** 2024; 11:1493447.
18. Banach M, Reiner Ž, Surma S, et al. 2023: The year in cardiovascular disease—the year of new and prospective lipid-lowering therapies. Arch Med Sci. 2023; 19:1469–1480.
19. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, et al; ESC Scientific Document Group. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2020;41(1):111-188.
20. Lan Y, Chen G, Wu D, Ding X, Huang Z, Wang X, et al. Temporal relationship between atherogenic dyslipidemia and inflammation and their joint cumulative effect on type 2 diabetes onset: a longitudinal cohort study. BMC Med. 2023; 21:31.