

Oxidative Stress and Inflammatory Biomarkers as Predictors of Cardiovascular Complications in Type 2 Diabetes Mellitus: A Clinical and Biochemical Evaluation

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ABSTRACT

Background: Cardiovascular disease (CVD) is a major cause of morbidity and mortality among individuals with Type 2 Diabetes Mellitus (T2DM). Oxidative stress and systemic inflammation are key mechanisms contributing to vascular dysfunction and cardiac complications. This study evaluates the predictive value of oxidative and inflammatory biomarkers in identifying cardiovascular risk among T2DM patients.

Methods: A cross-sectional study was conducted at Abwa Medical College, Faisalabad, and the University of Lahore Teaching Hospital, Lahore Pakistan, from March 2022 to May 2023. A total of 130 T2DM patients were enrolled and categorized into two groups: those with cardiovascular complications (n = 62) and those without (n = 68). Clinical data, biochemical parameters, oxidative stress markers malondialdehyde (MDA) and superoxide dismutase (SOD) and inflammatory biomarkers (hs-CRP, IL-6, TNF- α) were analyzed. Echocardiography and carotid intima-media thickness (CIMT) assessments were performed. Statistical analysis included t-tests, correlation analysis, and logistic regression.

Results: Patients with cardiovascular complications exhibited significantly higher MDA levels ($p < 0.001$), lower SOD activity ($p = 0.001$), and elevated inflammatory biomarkers including hs-CRP, IL-6, and TNF- α ($p < 0.001$). Echocardiographic findings revealed reduced ejection fraction and increased left ventricular mass index in the cardiovascular group ($p < 0.001$). MDA showed strong positive correlation with CIMT, while SOD demonstrated a significant negative correlation. Logistic regression identified elevated MDA, low SOD, high hs-CRP, and elevated IL-6 as independent predictors of cardiovascular complications in T2DM.

Conclusion: Oxidative stress and inflammatory biomarkers are strong predictors of cardiovascular complications in T2DM. Elevated MDA, reduced SOD activity, and increased hs-CRP and IL-6 correlate with structural and functional cardiac abnormalities. Integrating these biomarkers into clinical evaluation may enhance early cardiovascular risk detection and guide preventive strategies for diabetic patients.

Keywords: Type 2 diabetes mellitus, oxidative stress, inflammation, cardiovascular disease, MDA, SOD, hs-CRP, IL-6, TNF- α .

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) has emerged as one of the most prevalent chronic metabolic disorders worldwide, contributing substantially to long-term disability, reduced quality of life, and premature mortality. Its global incidence continues to rise due to urbanization, sedentary lifestyles, obesity, and aging populations^{1,2}. A major concern in T2DM is the markedly increased risk of cardiovascular complications, including coronary artery disease, myocardial infarction, heart failure, stroke, and peripheral vascular disease. These cardiovascular events account for more than 70% of diabetes-related deaths, highlighting the urgent need for early and accurate prediction of cardiovascular risk in diabetic individuals³.

The pathophysiology linking T2DM with cardiovascular disease (CVD) is multifactorial. Chronic hyperglycemia leads to the generation of excessive reactive oxygen species (ROS) through glucose autooxidation, mitochondrial dysfunction, and activation of the polyol and protein kinase C pathways⁴. When the production of ROS exceeds the capacity of the endogenous antioxidant defense system, a state of oxidative stress develops. Oxidative stress plays a pivotal role in endothelial dysfunction, lipid peroxidation, vascular inflammation, atherosclerotic plaque formation, and myocardial tissue injury⁵. Malondialdehyde (MDA), a principal marker of lipid peroxidation, is widely used to quantitatively assess oxidative damage in cardiometabolic disorders. Conversely, antioxidant enzymes such as superoxide dismutase (SOD) represent the body's protective mechanisms; diminished levels indicate

compromised oxidative defense⁶.

Alongside oxidative injury, inflammation serves as a critical driver of diabetic cardiovascular pathology. Low-grade systemic inflammation persists throughout the course of T2DM due to adipose tissue dysfunction, hyperglycemia-induced metabolic stress, and endothelial activation⁷. Circulating inflammatory biomarkers such as high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) are known to promote endothelial dysfunction, enhance plaque instability, and accelerate atherosclerosis. Elevated levels of these markers correlate strongly with adverse cardiovascular outcomes and may serve as early predictors of cardiometabolic deterioration⁸.

Given the interconnected roles of oxidative stress and inflammation, a combined assessment of oxidative and inflammatory biomarkers may provide more precise insight into cardiovascular risk among T2DM patients. Early identification of high-risk individuals through biochemical profiling can support timely therapeutic interventions, improve clinical monitoring, and reduce the burden of diabetic cardiovascular morbidity^{9,10}.

This study aims to evaluate the predictive significance of oxidative stress and inflammatory biomarkers in T2DM patients, comparing individuals with established cardiovascular complications to those without. By examining MDA, SOD, hs-CRP, IL-6, and TNF- α levels in relation to clinical cardiac parameters, the study seeks to enhance understanding of their diagnostic and prognostic value in diabetic cardiovascular disease¹¹.

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MATERIALS AND METHODS

This cross-sectional clinical and biochemical study was conducted at Abwa Medical College, Faisalabad, in collaboration with the Department of Medicine and The University of Lahore Teaching Hospital, Lahore. The study duration extended from March 2022 to May 2023. A total of 130 adult patients diagnosed with Type 2 Diabetes Mellitus (T2DM) were recruited using consecutive sampling from outpatient and inpatient medical units of both centers. The study population was divided into two groups: patients with clinically confirmed cardiovascular complications related to diabetes, and patients with T2DM but without overt cardiovascular involvement. Cardiovascular complications were defined based on standardized clinical criteria, echocardiographic findings, electrocardiographic changes, and documented history of myocardial infarction, ischemic heart disease, or left ventricular dysfunction.

All patients aged between 35 and 70 years with a confirmed diagnosis of T2DM for at least one year were eligible for inclusion. Individuals with type 1 diabetes, chronic kidney disease (stage 4–5), autoimmune disorders, active infections, malignancy, acute inflammatory conditions, or recent surgery within the past three months were excluded to minimize confounding effects on oxidative and inflammatory biomarkers. Demographic variables, medical history, duration of diabetes, medication use, smoking status, and anthropometric measurements were recorded through a structured proforma. Blood pressure and body mass index (BMI) were measured using standardized clinical protocols.

Venous blood samples were collected after an overnight fast of 10–12 hours. Samples were processed immediately in the Biochemistry Laboratory of Abwa Medical College. Serum and plasma were aliquoted and stored at -20°C until analysis. Oxidative stress biomarkers were determined through validated biochemical assays: malondialdehyde (MDA) levels were quantified using the thiobarbituric acid reactive substances (TBARS) method, while superoxide dismutase (SOD) activity was assessed via the inhibition of pyrogallol autoxidation. Inflammatory markers including high-sensitivity C-reactive protein (hs-CRP) were measured by immunoturbidimetric assay, and interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits following manufacturer protocols. Standard biochemical parameters such as fasting plasma glucose, HbA1c, lipid profile, and renal function tests were analyzed using automated clinical chemistry analyzers.

Cardiovascular assessment included resting electrocardiography (ECG) and two-dimensional echocardiography, performed by consultant cardiologists at SSMC. Echocardiographic evaluation included measurement of left ventricular ejection fraction (LVEF), left ventricular mass index (LVMI), diastolic function, and structural abnormalities. Carotid intima-media thickness (CIMT) was measured in a subset of patients when clinically indicated.

All laboratory assays were performed in duplicate to ensure accuracy. Internal quality control procedures were applied daily, and external quality assurance was maintained through participation in national proficiency testing programs. Data were entered and analyzed using SPSS version 26. Continuous variables were expressed as mean \pm standard deviation, while categorical variables were presented as frequencies and percentages. Independent sample t-tests were used to compare biomarker levels between groups, and Pearson correlation analysis was applied to determine associations between oxidative/inflammatory markers and cardiovascular parameters. Logistic regression was performed to identify independent predictors of cardiovascular complications among T2DM patients. A p-value <0.05 was considered statistically significant.

Ethical approval for the study was obtained from the Institutional Review Boards of Abwa Medical College, Faisalabad, and Sughra Shafi Medical Complex (SSMC), Pakistan. Informed written consent was obtained from all participants prior to

enrollment. Confidentiality and anonymity were maintained throughout the study.

RESULTS

A total of 130 patients with Type 2 Diabetes Mellitus were included in the study, of whom 62 (47.7%) had documented cardiovascular complications and 68 (52.3%) had no cardiovascular involvement. The mean age of the study population was 54.6 ± 8.9 years, and although age did not differ significantly between the groups, the duration of diabetes, BMI, systolic blood pressure, and HbA1c were significantly higher among patients with cardiovascular complications. These clinical and demographic details are summarized in Table 1, which shows that patients with cardiovascular disease had a longer history of diabetes ($p = 0.001$), higher BMI ($p = 0.004$), and poorer glycemic control ($p = 0.003$). The presence of elevated blood pressure in this group further reflected the metabolic and hemodynamic burden associated with cardiovascular pathology in T2DM (Table 1).

Oxidative stress markers displayed marked differences between the two groups. Patients with cardiovascular complications had significantly higher malondialdehyde (MDA) levels, indicating increased lipid peroxidation, while superoxide dismutase (SOD) activity was significantly reduced, showing impaired antioxidant defense. These findings are presented in Table 2, where MDA levels were nearly double in patients with cardiovascular involvement ($p < 0.001$), and SOD activity was significantly lower ($p = 0.001$). The imbalance between oxidative injury and antioxidant capacity suggests that oxidative stress plays a prominent role in the progression of cardiovascular damage in diabetic individuals.

Inflammatory biomarkers also exhibited a clear pattern of elevation in the cardiovascular group. High-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) were significantly higher in patients with cardiac complications. As shown in Table 3, hs-CRP levels were almost double in the cardiovascular group ($p < 0.001$), and both IL-6 and TNF- α were elevated significantly ($p < 0.001$ for both). This inflammatory profile reflects the persistent state of low-grade inflammation in T2DM and its contribution to vascular dysfunction, plaque instability, and myocardial impairment.

Cardiovascular structural and functional parameters also differed markedly between the two groups. Echocardiographic findings indicated that the mean left ventricular ejection fraction was significantly lower in patients with cardiovascular complications, while the left ventricular mass index and carotid intima-media thickness were substantially higher, indicating both functional impairment and structural remodeling. These findings are detailed in Table 4, where the differences in LVEF, LVMI, and CIMT all reached high statistical significance ($p < 0.001$). The increased CIMT further reflects subclinical atherosclerosis in the affected patients.

This figure 1 demonstrates the difference in oxidative stress levels between Type 2 Diabetes Mellitus patients with cardiovascular complications and those without. Patients with cardiovascular disease showed significantly higher MDA levels (6.8 nmol/mL) compared to those without complications (4.1 nmol/mL), indicating increased lipid peroxidation and oxidative stress in the cardiovascular group.

Correlation analysis revealed that MDA had a strong positive correlation with carotid intima-media thickness ($r = 0.61$, $p < 0.001$), whereas SOD activity was negatively correlated with both MDA ($r = -0.52$, $p < 0.001$) and CIMT ($r = -0.49$, $p < 0.001$), reinforcing the central role of oxidative stress in vascular injury. Inflammatory markers, particularly hs-CRP and IL-6, showed significant positive correlations with left ventricular mass index, while higher IL-6 and TNF- α levels were associated with lower ejection fraction, indicating inflammatory involvement in myocardial dysfunction. Logistic regression analysis demonstrated that elevated MDA, reduced SOD activity, high hs-CRP, and elevated IL-6 were independent predictors of cardiovascular complications,

even after adjusting for confounders such as duration of diabetes and LDL cholesterol. These findings highlight the predictive significance of oxidative and inflammatory biomarkers in identifying high-risk diabetic patients.

Table 1: Baseline Demographic and Clinical Characteristics (N = 130)

Variable	With CVD (n=62)	Without CVD (n=68)	p-value
Age (years)	55.4 ± 8.7	53.9 ± 9.1	0.29
Duration of T2DM (years)	10.6 ± 4.2	7.1 ± 3.9	0.001
BMI (kg/m ²)	29.7 ± 4.3	27.1 ± 3.8	0.004
Systolic BP (mmHg)	146 ± 17	132 ± 14	<0.001
HbA1c (%)	8.9 ± 1.2	7.8 ± 1.1	0.003

Table 2: Oxidative Stress Biomarkers

Biomarker	With CVD	Without CVD	p-value
MDA (nmol/mL)	6.8 ± 1.4	4.1 ± 1.2	<0.001
SOD (U/mL)	2.9 ± 0.8	4.4 ± 0.9	0.001

Table 3: Inflammatory Biomarker Levels

Biomarker	With CVD	Without CVD	p-value
hs-CRP (mg/L)	6.2 ± 1.9	3.1 ± 1.1	<0.001
IL-6 (pg/mL)	13.8 ± 4.2	8.4 ± 3.3	<0.001
TNF-α (pg/mL)	22.7 ± 5.1	14.3 ± 4.6	<0.001

Table 4: Echocardiographic and Vascular Parameters

Parameter	With CVD	Without CVD	p-value
LVEF (%)	48.2 ± 6.1	58.4 ± 3.9	<0.001
LVMI (g/m ²)	121 ± 18	101 ± 15	<0.001
CIMT (mm)	0.92 ± 0.18	0.68 ± 0.14	<0.001

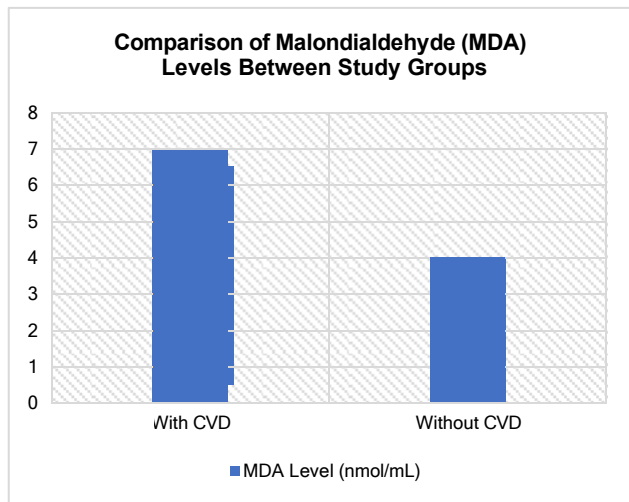


Figure 1: Comparison of Malondialdehyde (MDA) Levels Between Study Groups

DISCUSSION

The findings of this study demonstrate a significant association between oxidative stress, inflammatory biomarkers, and cardiovascular complications in patients with Type 2 Diabetes Mellitus¹². Patients with established cardiovascular disease exhibited markedly elevated levels of malondialdehyde (MDA) and significantly reduced superoxide dismutase (SOD) activity, reflecting an imbalance between pro-oxidant and antioxidant forces¹³. This imbalance represents a key pathological mechanism contributing to endothelial dysfunction, increased vascular stiffness, and accelerated atherosclerosis in diabetic individuals. Diabetes-induced hyperglycemia promotes excessive generation of reactive oxygen species through multiple pathways such as glucose autooxidation, mitochondrial dysfunction, and activation of the polyol pathway which collectively worsen oxidative stress and lead to tissue damage. The higher MDA levels observed in our

cardiovascular group are consistent with previous research identifying lipid peroxidation as a major contributor to cardiac structural deterioration, plaque instability, and reduced myocardial contractility^{14,15}.

Reduced antioxidant capacity, as evidenced by lower SOD activity, further reflects the disruption of physiological defenses required to neutralize reactive oxygen species. In our study, reduced SOD levels showed a significant negative correlation with carotid intima-media thickness and a positive association with increased cardiovascular burden¹⁶. This observation aligns with existing literature, which highlights that diminished antioxidant enzymes accelerate the progression of subclinical atherosclerosis and compromise myocardial function in diabetic patients. Collectively, these findings emphasize the critical role of oxidative stress as an early mediator and predictor of cardiovascular complications in T2DM¹⁷.

In addition to oxidative stress, this study highlights the strong contribution of systemic inflammation to cardiovascular risk. Inflammatory biomarkers including hs-CRP, IL-6, and TNF-α were significantly elevated in patients with cardiovascular disease, and each exhibited meaningful correlations with cardiac structural parameters such as left ventricular mass index and ejection fraction¹⁸. Chronic low-grade inflammation is a hallmark of T2DM, largely driven by adipose tissue dysfunction, altered insulin signaling, and endothelial activation. Elevated hs-CRP reflects hepatic inflammatory response and has been widely validated as a sensitive marker of early vascular injury. IL-6 and TNF-α contribute to progression of atherosclerosis by promoting endothelial adhesion molecule expression, increasing oxidative stress, and affecting lipid metabolism. Their elevation in our cardiovascular group and their association with reduced ejection fraction further highlight their role in cardiac dysfunction¹⁹.

The logistic regression analysis supports the prognostic utility of these biomarkers. Elevated MDA, reduced SOD, and increased hs-CRP and IL-6 were identified as independent predictors of cardiovascular complications, even after adjusting for traditional risk factors such as diabetes duration, LDL cholesterol, and blood pressure. This finding underscores the potential of incorporating oxidative and inflammatory biomarkers into routine clinical risk assessments. Such an approach could identify high-risk patients earlier, enabling timely therapeutic interventions and targeted monitoring strategies^{20,21}.

Our study findings complement existing scientific evidence but also provide meaningful clinical insight for local healthcare settings²². The dual burden of diabetes and cardiovascular disease is particularly high in South Asian populations due to genetic predisposition, dietary patterns, and lifestyle factors. Therefore, identifying biochemical predictors that allow early detection and risk stratification can significantly reduce morbidity and mortality. Incorporating these biomarkers in clinical practice may guide cardioprotective strategies including intensive glycemic control, lipid-lowering therapy, antioxidant supplementation, and anti-inflammatory interventions²³.

Although the study strengthens the evidence supporting the predictive value of oxidative and inflammatory markers, certain limitations must be acknowledged²⁴. The cross-sectional design restricts causal inference, and longitudinal studies are required to confirm predictive accuracy over time. Additionally, certain confounding factors such as dietary intake, physical activity, glycemic variability, and medication adherence were not deeply analyzed. Despite these limitations, the large sample size, use of standardized biomarker assays, and dual-center methodology add strength to the reliability of the findings²⁵.

CONCLUSION

This study demonstrates that oxidative stress and inflammatory biomarkers serve as powerful predictors of cardiovascular complications in patients with Type 2 Diabetes Mellitus. Elevated MDA and reduced SOD activity reflect enhanced lipid peroxidation and impaired antioxidant defenses, while increased levels of hs-

CRP, IL-6, and TNF- α indicate systemic inflammation driving vascular and myocardial damage. These biochemical alterations correlate strongly with echocardiographic and vascular changes, and independently predict cardiovascular disease in diabetic patients. Routine clinical assessment incorporating oxidative and inflammatory biomarkers may provide a more comprehensive evaluation of cardiovascular risk beyond traditional parameters. Early identification of high-risk patients can guide timely preventive strategies, optimize treatment decisions, and ultimately reduce the cardiovascular burden associated with T2DM.

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Authors' Contributions:

- **M.H.** conceived the study, supervised data collection, and reviewed the manuscript.
 - **U.R.** contributed to study design, patient recruitment, and clinical data acquisition.
 - **A.R.H.** performed biochemical analyses, laboratory work, and data interpretation.
 - **A.J.** contributed to statistical analysis and manuscript drafting.
 - **M.S.Z.K.S.** assisted in data collection, literature review, and manuscript editing.
 - **W.Q.** contributed to echocardiographic assessments and cardiovascular data verification.
 - **M.N.S.** assisted with data management, quality control, and final manuscript approval.
- All authors read and approved the final manuscript.

Conflict of Interest: The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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