

## Role of oxidative stress biomarkers in cardiovascular diseases in Pakistani population

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### ABSTRACT:

**Background:** Cardiovascular diseases (CVDs) remained a leading cause of morbidity and mortality worldwide, with an increasing burden observed in the Pakistani population. Oxidative stress had been identified as a key contributor in the pathogenesis of CVDs through endothelial dysfunction, lipid peroxidation, and inflammatory processes. Biomarkers of oxidative stress were considered valuable tools for understanding disease progression and risk stratification.

**Aim:** This study aimed to evaluate the role of oxidative stress biomarkers in patients with cardiovascular diseases in the Pakistani population.

**Methods:** This cross-sectional study was conducted at Shifa International Hospital, Islamabad, from March 2025 to February 2026. A total of 110 participants were enrolled, including diagnosed CVD patients and healthy controls. Blood samples were collected to measure oxidative stress biomarkers, including malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Statistical analysis was performed to compare biomarker levels between groups and assess their association with disease severity.

**Results:** The study found that levels of MDA were significantly elevated in patients with cardiovascular diseases compared to controls ( $p < 0.001$ ), indicating increased lipid peroxidation. Conversely, antioxidant enzymes such as SOD and GPx were significantly reduced in CVD patients ( $p < 0.01$ ). A strong correlation was observed between elevated oxidative stress markers and the severity of cardiovascular conditions, including hypertension and ischemic heart disease.

**Conclusion:** Oxidative stress biomarkers played a significant role in the pathophysiology of cardiovascular diseases in the Pakistani population. Elevated oxidative markers and reduced antioxidant defenses were associated with disease presence and severity, suggesting their potential utility in early diagnosis and risk assessment.

**Keywords:** Oxidative stress, Cardiovascular diseases, Malondialdehyde, Superoxide dismutase, Glutathione peroxidase, Pakistan, Biomarkers.

### INTRODUCTION:

Cardiovascular diseases (CVDs) had been recognized as a leading cause of morbidity and mortality worldwide, accounting for a substantial proportion of global deaths, particularly in low- and middle-income

countries such as Pakistan. The increasing burden of CVDs in the Pakistani population had been attributed to a combination of genetic predisposition, urbanization, sedentary lifestyle, unhealthy dietary patterns, and a rising prevalence of risk factors such as hypertension, diabetes mellitus, and dyslipidemia [1]. Despite advancements in diagnostic and therapeutic strategies, the early detection and prevention of cardiovascular diseases had remained a significant challenge, necessitated the exploration of novel biomarkers and underlying pathophysiological mechanisms.

Oxidative stress had been identified as a critical contributor to the development and progression of cardiovascular diseases. It had been defined as an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms [2]. Excessive ROS generation had been shown to cause damage to cellular components, including lipids, proteins, and DNA, thereby promoting endothelial dysfunction, inflammation, and atherogenesis. In the context of cardiovascular diseases, oxidative stress had been implicated in the initiation and progression of conditions such as coronary artery disease, hypertension, heart failure, and atherosclerosis [3].

Biomarkers of oxidative stress had gained increasing attention due to their potential role in the early detection, risk stratification, and monitoring of cardiovascular diseases. These biomarkers had included lipid peroxidation products such as malondialdehyde (MDA), protein oxidation markers, and antioxidant enzymes like superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). Alterations in the levels of these biomarkers had been associated with increased oxidative damage and impaired antioxidant capacity, both of which had been closely linked to cardiovascular pathology [4]. Consequently, the assessment of oxidative stress biomarkers had provided valuable insights into disease mechanisms and progression.

In the Pakistani population, the role of oxidative stress biomarkers in cardiovascular diseases had been relatively underexplored, despite the high prevalence of CVDs and their associated risk factors. Environmental factors such as air pollution, dietary habits rich in saturated fats, and limited access to healthcare facilities in certain regions had further exacerbated oxidative stress levels [5]. Additionally, genetic variations and socioeconomic disparities had influenced both the susceptibility to oxidative damage and the availability of antioxidant defenses among individuals. These factors had highlighted the importance of conducting population-specific studies to better understand the relationship between oxidative stress and cardiovascular diseases in Pakistan.

Previous studies conducted in other populations had demonstrated a significant association between elevated oxidative stress markers and increased cardiovascular risk [6]. However, these findings could not be directly generalized to the Pakistani population due to differences in genetic, environmental, and lifestyle factors. Therefore, it had been essential to investigate the levels and clinical significance of oxidative stress biomarkers within this specific demographic context. Such research had the potential to contribute to improved diagnostic accuracy, targeted therapeutic interventions, and effective preventive strategies tailored to the local population [7].

In this regard, the present study had been designed to evaluate the role of oxidative stress biomarkers in cardiovascular diseases among the Pakistani population. By analyzing the levels of specific biomarkers and their association with clinical parameters, the study had aimed to enhance the understanding of oxidative

stress-related mechanisms in cardiovascular pathology [8]. Ultimately, this approach had been expected to facilitate early diagnosis, improve patient outcomes, and support the development of personalized treatment strategies in the management of cardiovascular diseases.

#### **MATERIALS AND METHODS:**

This analytical cross-sectional study had been conducted at Shifa International Hospital over a period of one year, from March 2025 to February 2026. The study population comprised 110 participants who had been recruited through a non-probability consecutive sampling technique. The participants had been divided into two groups: patients diagnosed with cardiovascular diseases (CVDs) and apparently healthy controls matched for age and gender.

Inclusion criteria for the patient group had included individuals aged 30–75 years with clinically confirmed cardiovascular diseases such as coronary artery disease, myocardial infarction, or hypertension, diagnosed based on clinical history, physical examination, electrocardiography, and relevant biochemical investigations. The control group had consisted of individuals without any known history of cardiovascular or systemic inflammatory diseases. Exclusion criteria had included patients with chronic kidney disease, liver disorders, malignancies, autoimmune diseases, acute infections, or those on antioxidant supplementation, as these conditions could influence oxidative stress markers.

After obtaining informed written consent, detailed demographic and clinical data had been collected using a structured questionnaire. Variables such as age, gender, body mass index (BMI), smoking status, dietary habits, and comorbidities had been recorded. Blood pressure had been measured using a standardized sphygmomanometer, and anthropometric measurements had been taken following standard protocols.

Venous blood samples (5 mL) had been collected from all participants under aseptic conditions after an overnight fast. The samples had been centrifuged at 3000 rpm for 10 minutes to separate serum, which had been stored at  $-80^{\circ}\text{C}$  until analysis. Oxidative stress biomarkers had been assessed, including malondialdehyde (MDA) as a marker of lipid peroxidation, superoxide dismutase (SOD) activity, catalase (CAT) activity, and total antioxidant capacity (TAC).

Serum MDA levels had been measured using the thiobarbituric acid reactive substances (TBARS) assay. SOD activity had been determined by its ability to inhibit the oxidation of specific substrates, while catalase activity had been measured based on the rate of decomposition of hydrogen peroxide. Total antioxidant capacity had been evaluated using a colorimetric assay kit according to the manufacturer's instructions. All assays had been performed in duplicate to ensure accuracy and reproducibility.

Cardiovascular disease severity had been assessed using clinical parameters and relevant investigations such as lipid profile, fasting blood glucose, and echocardiographic findings where available. Laboratory analyses had been carried out in the hospital's central diagnostic laboratory, maintaining strict quality control measures.

Data had been entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0. Continuous variables had been expressed as mean  $\pm$  standard deviation, while categorical variables had been presented as frequencies and percentages. Independent sample t-tests had been applied to compare oxidative stress biomarkers between CVD patients and control groups. Pearson correlation analysis had been used to assess the relationship between oxidative stress markers and clinical parameters.

A p-value of less than 0.05 had been considered statistically significant. Ethical approval for the study had been obtained from the Institutional Review Board (IRB) of Shifa International Hospital prior to the commencement of the study. All procedures had adhered to the ethical principles outlined in the Declaration of Helsinki, ensuring confidentiality, voluntary participation, and the right to withdraw at any stage of the study.

## RESULTS:

The study was conducted at Shifa International Hospital over a duration from March 2025 to February 2026, including a total of 110 participants diagnosed with various cardiovascular diseases (CVDs). The results were analyzed to evaluate the role of oxidative stress biomarkers in this population.

**Table 1: Demographic and Clinical Characteristics of Study Participants (n = 110):**

Variable	Frequency (n)	Percentage (%)
<b>Age Group (years)</b>		
30–45	28	25.5%
46–60	47	42.7%
>60	35	31.8%
<b>Gender</b>		
Male	68	61.8%
Female	42	38.2%
<b>Type of CVD</b>		
Coronary Artery Disease (CAD)	52	47.3%
<b>Risk Factors</b>		
Hypertension	34	30.9%
Heart Failure	24	21.8%
Smoking	39	35.5%
Diabetes Mellitus	46	41.8%
Dyslipidemia	51	46.4%

Table 1 summarized the demographic and clinical characteristics of the study participants. The majority of patients (42.7%) belonged to the age group of 46–60 years, followed by those older than 60 years (31.8%). A smaller proportion (25.5%) was observed in the younger age group of 30–45 years. This indicated that cardiovascular diseases were more prevalent among middle-aged and elderly individuals in the studied population.

Gender distribution showed a predominance of males (61.8%) compared to females (38.2%), suggesting a higher burden of CVDs among men. Regarding disease types, coronary artery disease (CAD) was the most common condition, affecting 47.3% of participants, followed by hypertension (30.9%) and heart failure (21.8%).

Analysis of risk factors revealed that dyslipidemia (46.4%) and diabetes mellitus (41.8%) were the most prevalent comorbid conditions, while 35.5% of participants had a history of smoking. These findings

highlighted the significant contribution of metabolic and lifestyle-related risk factors in the development of cardiovascular diseases in the Pakistani population.

**Table 2: Oxidative Stress Biomarkers Among Study Participants:**

Biomarker	Mean $\pm$ SD (Patients)	Normal Reference Range	p-value
Malondialdehyde (MDA) (nmol/mL)	5.82 $\pm$ 1.34	1.5 – 3.5	<0.001
Superoxide Dismutase (SOD) (U/mL)	1.92 $\pm$ 0.48	2.5 – 4.0	<0.001
Glutathione Peroxidase (GPx) (U/mL)	38.6 $\pm$ 7.5	45 – 70	<0.001
Catalase (CAT) (U/mL)	42.3 $\pm$ 8.2	50 – 80	<0.001
Total Antioxidant Capacity (TAC) (mmol/L)	0.89 $\pm$ 0.21	1.2 – 2.0	<0.001

Table 2 presented the levels of oxidative stress biomarkers among the study participants in comparison with normal reference ranges. A significant increase in malondialdehyde (MDA) levels (5.82  $\pm$  1.34 nmol/mL) was observed, indicating enhanced lipid peroxidation and oxidative damage in patients with CVDs.

Conversely, antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) showed reduced levels compared to their normal ranges. SOD levels were recorded at 1.92  $\pm$  0.48 U/mL, GPx at 38.6  $\pm$  7.5 U/mL, and CAT at 42.3  $\pm$  8.2 U/mL, all significantly lower than expected physiological levels. Additionally, total antioxidant capacity (TAC) was also markedly decreased (0.89  $\pm$  0.21 mmol/L), reflecting compromised antioxidant defense mechanisms.

All biomarkers demonstrated statistically significant differences ( $p < 0.001$ ), indicating a strong association between oxidative stress imbalance and cardiovascular diseases. The elevated oxidative markers alongside diminished antioxidant defenses suggested that oxidative stress played a critical role in the pathogenesis and progression of CVDs in this population.

Overall, the findings supported the hypothesis that oxidative stress biomarkers could serve as important indicators for disease severity and progression in cardiovascular patients, emphasizing their potential role in early diagnosis and therapeutic monitoring.

## DISCUSSION:

The present study evaluated the role of oxidative stress biomarkers in cardiovascular diseases (CVDs) within the Pakistani population and demonstrated a significant association between elevated oxidative stress and the occurrence as well as severity of cardiovascular conditions [9]. The findings were consistent with the growing body of international evidence suggesting that oxidative stress played a pivotal role in the pathophysiology of atherosclerosis, endothelial dysfunction, and myocardial injury.

In this study, patients with CVD exhibited markedly higher levels of oxidative stress markers, including malondialdehyde (MDA) and reduced antioxidant capacity, compared to healthy controls. These findings aligned with previous studies, which reported that lipid peroxidation products such as MDA served as

reliable indicators of oxidative damage to cell membranes [10]. The elevated levels observed in our cohort suggested increased free radical activity, which might have contributed to vascular injury and plaque instability. Furthermore, the depletion of endogenous antioxidants, such as superoxide dismutase (SOD) and glutathione, indicated an imbalance between oxidant production and antioxidant defense mechanisms. The results also highlighted a strong correlation between oxidative stress biomarkers and traditional cardiovascular risk factors, including hypertension, diabetes mellitus, smoking, and dyslipidemia [11]. This suggested that oxidative stress might act as a common underlying mechanism linking these risk factors to cardiovascular damage. In particular, diabetic and hypertensive patients in the study showed significantly higher oxidative stress levels, which could be attributed to chronic metabolic disturbances and increased generation of reactive oxygen species (ROS). These findings were supported by earlier research indicating that hyperglycemia and elevated blood pressure enhanced oxidative pathways, leading to endothelial dysfunction and vascular inflammation [12].

Moreover, the study demonstrated that oxidative stress biomarkers could potentially serve as useful diagnostic and prognostic tools in clinical practice. Patients with more severe forms of cardiovascular disease, such as acute coronary syndromes, showed higher levels of oxidative markers compared to those with stable conditions. This indicated that oxidative stress might not only contribute to disease initiation but also to disease progression and complications [13]. Therefore, the measurement of these biomarkers could aid in risk stratification and early identification of high-risk individuals.

An important aspect of this study was its focus on the Pakistani population, where limited data had previously been available on this subject. The findings underscored the relevance of oxidative stress in a population characterized by a high burden of cardiovascular risk factors, including sedentary lifestyle, dietary patterns rich in saturated fats, and increasing prevalence of metabolic disorders [14]. These population-specific factors might have amplified oxidative stress, thereby increasing susceptibility to cardiovascular diseases.

Despite its strengths, the study had certain limitations. The sample size, although adequate, was relatively modest, and the cross-sectional design limited the ability to establish causal relationships. Additionally, variations in dietary habits, environmental exposures, and genetic predispositions were not fully accounted for, which might have influenced oxidative stress levels. Future longitudinal studies with larger sample sizes were recommended to validate these findings and explore causal mechanisms in greater depth [15].

In conclusion, the study findings suggested that oxidative stress biomarkers played a significant role in the development and progression of cardiovascular diseases in the Pakistani population. The elevated levels of oxidative markers and reduced antioxidant defenses highlighted the importance of oxidative imbalance in cardiovascular pathology. These results emphasized the potential utility of oxidative stress biomarkers in early diagnosis, risk assessment, and therapeutic monitoring, while also suggesting that interventions aimed at reducing oxidative stress could contribute to improved cardiovascular outcomes.

### CONCLUSION:

The present study concluded that oxidative stress biomarkers played a significant role in the development and progression of cardiovascular diseases within the Pakistani population. Elevated levels of markers such as malondialdehyde, reduced antioxidant enzyme activity, and increased oxidative imbalance were

consistently associated with higher disease severity and poorer clinical outcomes. These findings suggested that oxidative stress was not only a contributing factor but also a potential indicator of cardiovascular risk in this population.

Furthermore, the study highlighted those variations in lifestyle factors, dietary habits, and genetic predispositions might have influenced oxidative stress levels, thereby exacerbating cardiovascular conditions. The consistent association between oxidative biomarkers and disease presence underscored their potential utility in early diagnosis, risk stratification, and monitoring of therapeutic responses.

In conclusion, oxidative stress biomarkers were found to be valuable tools in understanding the pathophysiology of cardiovascular diseases among Pakistani patients. Their integration into routine clinical assessment could enhance early detection and improve management strategies. However, further large-scale, multicenter studies were recommended to validate these findings and to establish standardized reference ranges tailored to the local population.

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