

SJMLS -9(2) - 019

Evaluation of 8-Hydroxy-2'-Deoxyguanosine, Interleukin-8 and Malondialdehyde Among Patients with Cardiovascular Diseases in Ibadan, Oyo State.Moronkeji A.I.*¹, Olaniyan M.F.¹

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<https://dx.doi.org/10.4314/sokjmls.v9i2.19>**Abstract**

Cardiovascular diseases (CVDs) are a leading cause of mortality and morbidity worldwide, with various risk factors contributing to their development and progression. Among these risk factors, oxidative stress and inflammation play crucial roles in the pathogenesis of CVDs. This study evaluated the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), interleukin-8 (IL-8), and malondialdehyde (MDA) among cardiac patients and explored their potential as biomarkers for cardiac conditions. This case-control study enrolled 121 cardiac patients from the Cardiology Unit of University College Hospital and controls from Ibadan metropolis. Demographic and clinical data were collected, and blood samples were obtained to measure serum levels of 8-OHdG, IL-8, and MDA using ELISA techniques. Correlations between biomarker levels were analyzed using student t-test. Patients with CVD conditions IL-8, and MDA compared with healthy controls. In this study, the Control group showed mean values of 0.09 ng/ml \pm 0.01 for 8-OHdG, 55.50 pg/ml \pm 2.06 for IL-8, and 1.21 ng/ml \pm 0.10 for MDA, the CVD patients group exhibited markedly higher mean values for 8-OHdG (0.416 ng/ml \pm 0.11), IL-8 (100.84 pg/ml \pm 4.91), and MDA (2.64 ng/ml \pm 0.11) with p-values 8-OHdG (P=0.0021), IL-8 (P=0.001) and MDA (P=0.001) in CVD patients compared to healthy controls, indicating heightened oxidative stress and inflammation in the test group. The strong positive correlations observed among the three biomarkers suggest shared underlying mechanisms in the development of CVDs. These findings underscore the potential of these

biomarkers as diagnostic or prognostic tools for cardiovascular diseases, offering insights into early detection, risk stratification, and treatment monitoring to improve patient outcomes.

Keywords: 8-hydroxy-2'-deoxyguanosine, interleukin-8, malondialdehyde, cardiovascular diseases, biomarkers, oxidative stress, inflammation

Introduction

Cardiovascular disease (CVD) ranks among the primary causes of mortality and morbidity on a global scale. Its development involves a multifaceted interplay of various factors. Disease onset isn't solely attributable to either genetic or environmental influences in isolation. Atherosclerosis serves as the primary underlying factor in CVD, marked by the presence of cellular debris, foam cells and activated immune cells containing oxidized low-density lipoprotein within arterial lesions (Frostegard *et al.*, 1999; Frostegard, 2013). Environmental exposure stands as a significant yet often underestimated risk element influencing the onset and severity of cardiovascular disease (CVD) (Cosselman *et al.*, 2015). The disease's prevalence among populations, families, and individuals is closely related to the interaction of many genes that are susceptible and environmental exposures. These interactions become integrated through dynamic, epigenetic regulatory systems, which ultimately result in the phenotype of the disease. (Sing *et al.*, 1995; Dennis, 2003; Gottesman and Gould, 2003). The frequency of risk factors for cardiovascular disease (CVD) is rising in developing nations

globally (Oguanobi *et al.*, 2013). Worldwide, cardiovascular disease (CVD) is responsible for the majority of chronic disease-related deaths, with approximately 17.9 million fatalities reported annually (Paradis and Chiolero, 2011). Furthermore, more than 80% of the world's CVD cases are found in countries with low or middle incomes. (Oguoma *et al.*, 2015). Heart and blood vessel abnormalities fall under the category of cardiovascular diseases, which includes conditions like coronary heart disease, cerebrovascular disease, rheumatic heart disease and other related conditions (Tunçay *et al.*, 2021). Heart attacks and strokes account for more than four-fifths of CVD-related deaths, with one-third of these deaths occurring in those under the age of 70 before their time. It is commonly known that a number of etiological risk factors influence the development of cardiovascular diseases (CVDs). In epidemiological research, these risk factors collectively account for over 90% of the risks associated with CVDs and include physical inactivity, obesity, diabetes, smoking and hypertension (Flora *et al.*, 2019). In Nigeria, limited reports exist regarding the association between socio-economic status and cardiometabolic syndrome, with varying prevalence of cardiometabolic risk factors observed between high and low socio-economic status groups (Adedoyin *et al.*, 2005; Mbada *et al.*, 2009; Adedoyin *et al.*, 2013). Oguanobi *et al.* (2013) highlighted that cardiovascular diseases (CVDs) were significant contributors to morbidity within the studied environment. They emphasized the importance of early detection, enhanced outpatient care, and the implementation of suitable secondary prevention programs in patient management, particularly at the community level. These measures are recommended to mitigate complications and decrease the necessity for hospital admissions. Despite the high fatality rate associated with cardiovascular diseases (CVDs), the proactive identification and prevention of underlying risk factors can notably diminish the global CVD epidemic. In addition to adopting healthier lifestyle practices, primary strategies for preventing and treating CVDs encompass the use of lipid-lowering medications, antihypertensives, antiplatelet agents, and anticoagulants. However, despite their effectiveness, substantial gaps persist in the treatment of CVDs (Flora *et al.*, 2019).

Ceasing tobacco use, consuming less salt, eating more fruits and vegetables, exercising frequently, and abstaining from excessive alcohol have all been shown to reduce the probability of cardiovascular disease. It is crucial for health policies to establish conditions that enable people to afford and easily choose healthy options, thereby motivating people to adopt and sustain healthy behaviors (Oguoma *et al.*, 2015). The diagnosis and management of cardiac diseases continue to pose significant challenges in healthcare, with a rising incidence of cardiac-related morbidity and mortality among the population. In the Cardiology unit of University College Hospital, Ibadan, there is a pressing need to address the lack of comprehensive biomarker-based assessments for newly diagnosed cardiac patients. This gap in knowledge and clinical practice highlights a critical problem: the absence of a thorough evaluation of 8-Hydroxy-2'-Deoxyguanosine (8-OHdG), Interleukin-8 (IL-8), and Malondialdehyde (MDA) as potential biomarkers for cardiac diseases. Despite their known associations with oxidative stress, inflammation, and cellular damage, there is a dearth of systematic studies examining their utility in predicting, diagnosing, or monitoring cardiac conditions in this specific patient population. Consequently, this study aims to address this problem by conducting a comprehensive evaluation of these biomarkers among newly diagnosed cardiac patients at University College Hospital, Ibadan, with the ultimate goal of improving early detection, prognosis, and treatment strategies for cardiac diseases.

Materials and Method

Study Area

The majority of the population of Ibadan, which is in Oyo State, Nigeria, speaks Yoruba. At 3,080 square kilometers, it's said to be the biggest indigenous metropolis in Nigeria. 1,889,776 people live in the municipality's five local government districts, while 1,413,081 people live in the surrounding villages. It is a more urbanized and metropolitan metropolis rather than one from a distant past. It stands out from other Yoruba cities due to its size. The name of several wards of Ibadan, including Oke-Ado, Oke-Foko, Oke-Sapati, Oke-Are, Oke-Oluokun, and Oke-Bola, comes from the city's many hills,

or Oke. The co-ordinate of the region is 7.3775° N, 3.9470° E.

Ethical Approval

The University of Ibadan and the University College Hospital, Ibadan (UI/UCH) Ethics Review Committee granted ethical approval for this project, with assigned number UI/EC/23/0741.

Study Design

This study is a case-control study alongside epidemiological survey of patients with newly diagnosed cardiovascular disease attending the Medical Outpatient Clinic, Cardiology Unit, UCH, Ibadan.

Inclusion Criteria: Samples were obtained from all known cardiac subjects who gave informed consent.

Exclusion Criteria: Known cardiac Individuals of the required age bracket, but do not consent to participate in the research. Also, pregnant women were excluded from participation in the study.

Study Population

One hundred and twenty-one (121) individuals who were newly diagnosed for various cardiovascular diseases were selected from the Medical Outpatient Clinic, Cardiology Unit, Medical Outpatient Clinic, University College Hospital, Ibadan. Controls included healthy individuals selected within Ibadan metropolis.

Data Collection

Data was obtained from all healthy subjects who gave informed consent. A history of previous and present diseases (hypertension, cardiac disease, chronic heart failure, arrhythmia, cerebrovascular disease, and malignancies) as well as any relevant information were gathered.

All subjects had at least five minutes of rest before having their diastolic and systolic blood pressures (DBP and SBP) monitored. Participants who filled out their consent to participate in the research through one-on-one forms were asked to come to University College Hospital, Ibadan for peripheral blood collection.

Specimen Collection

Blood Sample

Participants had a venipuncture of the median cubital vein of the antecubital fossa using a BD vacutainer to extract 5 ml of blood, which was then centrifuged for 12 minutes at 1200 RPM in a lithium heparin bottle. Prior to the experiment, the aliquots were kept in cryogenic tubes at -80°C.

Laboratory Analysis

Determination of 8-hydroxydeoxyguanosine (8-OHdG) Concentration

The determination of 8-hydroxydeoxyguanosine (8-OHdG) was carried out using the 8-OHdG ELISA kit (Cell Biolabs, San Diego, CA, USA).

Determination of Malondialdehyde Concentration

The concentration of human MDA concentration was determined using MDA ELISA kit (Kamiya Biomedical Company, Seattle, USA).

Determination of IL-8 Concentration

Commercially available ELISA kit (Platinum ELISA, eBioscience, Waltham, MA, USA) was used to test human serum for the proinflammatory cytokine IL-8, strictly adhering to the manufacturer's instructions.

Result

Table 1 shows the socio-demographic characteristics of the study participants.

Table 1: Socio-demographic characteristics of participants

Socio-demographic characteristics	Test Group (n=101)	Control Group (n=20)
Age		
31-40 years	10 (9.9%)	2 (10.0%)
41-50 years	15 (14.9%)	5 (25.0%)
51-60 years	31 (30.6%)	5 (25.0%)
61-70 years	45 (44.6%)	8 (40.0%)
Marital Status		
Single	40 (39.6%)	8 (40.0%)
Married	61 (60.4%)	12 (60.0%)
Religion		
Christian	60 (59.4%)	8 (40.0%)
Muslim	41 (40.6%)	12 (60.0%)
Ethnicity		
Yoruba	55 (54.5%)	11 (55.0%)
Other	46 (45.5%)	9 (45.0%)
Gender		
Male	25(25%)	11 (55.0%)
Female	76 (75%)	9(45.0%)
Smoking History		
Yes	16 (16%)	1 (5.0%)
No	85(84%)	19 (95.0%)
Alcohol Consumption		
No	53 (52%)	19 (95.0%)
Occasionally	13 (13%)	1(5%)
Rarely	6 (6%)	
Usually	29 (29%)	

In this study, the control group showed mean values of 0.09 ng/ml ± 0.01 for 8-OHdG, 55.50 pg/ml ± 2.06 for IL-8, and 1.21 ng/ml ± 0.10 for MDA. In contrast, the cardiac patients group exhibited markedly higher mean values for 8-OHdG (0.416 ng/ml ± 0.11), IL-8 (100.84 pg/ml ± 4.91), and MDA (2.64 ng/ml ± 0.11) as presented in Table 2.

Table 2: Mean and standard deviation of the values of plasma 8-OHdG, IL-8 and MDA obtained in the Control and test subjects

Parameter	Control (N=20)	CVD Patients (N=101)
8-ohdg (ng/ml)	0.09 ± 0.01	0.416 ± 0.11
IL-8 (pg/ml)	55.50 ± 2.06	100.84 ± 4.91
MDA (ng/ml)	1.21 ± 0.10	2.64 ± 0.11

Table 3 presents the results of statistical analyses (t-test and p-value) comparing the plasma levels of 8-ohdG, IL-8 and MDA between control subjects and the CVD patients. The "t" values for 8-OHdG, IL-8, and MDA were 2.977, 30.11, and 49.45, respectively, while the associated p-values of 0.002108** for 8-OHdG and 0.00001 for IL-8 and MDA, indicating statistical significance. There was a significantly higher values of 8-OHdG, IL-8 and MDA in Cardiac Patients than in Control subjects (P< 0.05) suggesting potential associations between these parameters and the presence of cardiac diseases.

Table 3: Comparative analysis of the plasma 8-OHDG, IL-8 and MDA obtained in the Control and Test Subjects

Parameters		Control vs CVD Patients
8-ohdg(ng/ml)	“t”	2.977
	“p”	0.0021**
il-8 (pg/ml)	“t”	30.11
	“p”	.0001**
MDA (ng/ml)	“t”	49.45
	“p”	.0001**

** Significant 8-OHDG, IL-8 and MDA

Table 4 presents the Pearson correlation coefficients and corresponding p-values for the relationships among the three key variables: 8-hydroxy-2 -deoxyguanosine (8-OHdG), interleukin-8 (IL-8), and malondialdehyde (MDA). There was a significantly strong correlation between 8-OHdG, IL-8 and MDA.

8- OHdG and IL-8: There is a Correlation coefficient (r) (0.6404) indicating a moderately positive correlation between 8-OHdG and IL-8 with p-value = 0.0001** which is highly statistically significant. As 8-OHdG level increases, IL-8 increases as well.

8-OHdG and MDA: There is a positive correlation between 8-OHdG and MDA (0.92) suggesting a strong positive relationship with p-value= 0.0001** which is statistically significant. As 8- ohdg level increases, MDA increases as well.

IL-8 and MDA: There is a positive correlation between 8-ohdg and MDA (0.9123) suggesting a strong positive relationship with p-value= 0.0001** which is statistically significant. As IL-8 level increases, MDA increases as well.

This table shows that there are strong positive correlations among the plasma levels of 8-OHdG, IL-8, and MDA in the study participants. High levels of one parameter are associated with high levels of the other two parameters. These strong correlations suggest potential interconnections or shared underlying mechanisms among these variables in the study.

Table 4: Pearson Correlation Coefficient among variables

Parameters		8-OHdG (ng/ml)	IL-8 (pg/ml)	MDA (ng/ml)
8- OHdG (ng/ml)	“r”	1	0.6404	0.92
	“p”	—	0.00001**	0.00001**
IL-8 (pg/ml)	“r”	0.6404	1	0.9123
	“p”	0.00001**	—	0.00001**
MDA (ng/ml)	“r”	0.92	0.9123	1
	“p”	0.00001**	0.00001**	—

**Significant correlation

Discussion

Oxidative stress, inflammation and lipid peroxidation have been implicated as crucial pathophysiological mechanisms in the development and the progression of CVDs, including myocardial infarction, coronary artery disease and heart failure (Mangge *et al.*, 2014; Siti *et al.*, 2015; Zhang *et al.*, 2018).

The study presented evaluates the levels of 8-OHdG, IL-8 and MDA among cardiac patients attending the University College Hospital (UCH) in Ibadan, Nigeria. The results reported clearly demonstrate significantly higher levels of these biomarkers in the test subjects compared to the control subjects.

Specifically, the mean plasma levels of 8-OHdG, IL-8 and MDA were 0.416 ± 0.11 ng/mL, 100.84 ± 4.91 pg/mL, and 2.64 ± 0.11 ng/mL, respectively, in the subject group, compared to 0.09 ± 0.01 ng/mL, 55.50 ± 2.06 pg/mL, and 1.21 ± 0.10 ng/mL, respectively, in the control subjects. These differences were significant statistically, with p-values of 0.002108^{**} ($P < 0.05$) for 8-OHdG and $P < 0.00001$ for IL-8 and MDA.

Elevated levels of 8-OHdG observed in the cardiac patients align with the findings from numerous studies (Jaruga *et al.*, 2010 and Kobayashi *et al.*, 2015) which reported increased oxidative DNA damage, as reflected by higher 8-OHdG levels, in patients with coronary artery disease, heart failure and myocardial infarction. Oxidative stress plays a crucial role in pathogenesis of CVDs, contributing to endothelial dysfunction, inflammatory processes, and plaque formation (Madamanchi *et al.*, 2005; Förstermann, 2008). Research has consistently demonstrated elevated circulating 8-OHdG levels with coronary artery disease (CAD) and associated major adverse cardiovascular events (MACE) patients. For instance, Xiang *et al.* (2015) observed a significantly higher serum levels of 8-OHdG in patients with CAD compared to healthy controls. Elevated levels of 8-OHdG indicate an increase in oxidative stress and have been associated with various cardiovascular diseases (CVD), although the precise mechanisms linking 8-OHdG to CVD pathology remain incompletely

understood (Liu *et al.*, 2019). Research has consistently shown that the elevated 8-OHdG levels are associated with factors contributing to cardiovascular risk, such as smoking, diabetes, hypertension, and dyslipidemia (Wu *et al.*, 2004). Furthermore, increased 8-OHdG levels have been linked to worsened cardiac remodeling, atherogenesis, arrhythmias, and mortality risk in conditions like ischemic-heart disease and heart failure (Andreassi *et al.*, 2013). The observed elevation in 8-OHdG levels in the cardiac patients suggests increased oxidative stress and DNA damage, which may contribute to development and progression of cardiovascular conditions.

The significantly higher levels of IL-8 in the test group as compared to the control group are consistent with previous studies (Zernecke *et al.*, 2008 and Shetelig *et al.*, 2018) which demonstrated the involvement of IL-8 in pathogenesis of atherosclerosis and myocardial infarction. Elevated IL-8 levels correlate with type 2 diabetes mellitus and insulin resistance risk (Kim *et al.*, 2019) and its involvement extends to metabolic disorders like obesity, diabetes, and dyslipidemia, well-established CVD risk factors (Virani *et al.*, 2021). IL-8 promotes the recruitment of inflammatory cells like monocytes and neutrophils, to the site of atherosclerotic plaques, contributing to plaque progression and destabilization (Zernecke *et al.*, 2008; Apostolakis *et al.*, 2009). Additionally, in the context of acute myocardial infarction, IL-8 plays a role in the inflammatory response, contributing to neutrophil recruitment, tissue damage, and impaired myocardial recovery (Shetelig *et al.*, 2018). According to Apostolakis *et al.* (2009) the elevated IL-8 levels observed in the cardiac patients may reflect ongoing inflammatory processes and could potentially serve as a biomarker for assessing the severity or prognosis of their cardiovascular conditions.

Elevated levels of MDA in the test group are consistent with previous studies (Harangi *et al.*, 2002 and Zhang *et al.*, 2018) which reported elevated MDA levels in patients with myocardial infarction, coronary artery disease, and heart failure. MDA is a reliable indicator of lipid peroxidation, which contributes to the

pathogenesis of atherosclerosis by promoting endothelial dysfunction, foam cell formation, and plaque instability (Salvayre *et al.*, 2002; Stocker & Keaney, 2004). Elevated MDA levels signify augmented cardiovascular risks, and it is associated with adverse outcomes such as myocardial tissue damage and worsened cardiac function, spanning various cardiovascular disease (CVD) presentations (Martín-Fernández *et al.*, 2009; Giera *et al.*, 2012). Elevated MDA levels correlate with disease severity and progression across these conditions, implicating its involvement in adverse cardiovascular outcomes (Ayala *et al.*, 2014; Tsikas *et al.*, 2022). Factors like dietary habits, smoking, and environmental exposures can influence MDA measurements, warranting cautious interpretation (Ayala *et al.*, 2014; Gaschler & Stockwell, 2017). The observed elevation in MDA levels in the cardiac patients suggests increased oxidative stress and lipid peroxidation, which may play a role in the development and progression of their cardiovascular condition.

Conclusion

The study assessed the levels of malondialdehyde (MDA), interleukin-8 (IL-8), and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in cardiac patients visiting University College Hospital (UCH) in Ibadan, Nigeria. The results showed that cardiac patients had much higher levels of these markers than did healthy controls, suggesting that people with cardiovascular disorders have higher levels of oxidative stress, inflammation, and lipid peroxidation. Increased 8-OHdG levels are indicative of reactive oxygen species-induced DNA damage, which can aid in the onset and advancement of cardiovascular diseases. Likewise, elevated levels of IL-8 signify an intensified inflammatory reaction, which is recognized as a risk factor for cardiovascular disorders. Increased MDA levels in heart patients are a sign of lipid peroxidation, which is connected to atherosclerosis and oxidative stress. Strong positive correlations between the levels of all three biomarkers were also found in the study, indicating the possibility of shared underlying mechanisms or links in the pathophysiology of cardiovascular illnesses. The results demonstrate the potential usefulness of these biomarkers in determining the degree of inflammation, oxidative

stress, and lipid peroxidation in cardiac patients. This information may help with the early diagnosis, prognosis, and treatment of cardiovascular disorders.

Recommendation:

The following recommendations can be made in light of the study's findings:

- Incorporate the measurement of 8-OHdG, IL-8 and MDA levels into the routine diagnostic workup for cardiac patients, as these biomarkers can provide valuable insights into the underlying oxidative stress, inflammatory processes, and lipid peroxidation.
- Utilize the levels of these biomarkers, in conjunction with other clinical parameters, to stratify cardiac patients based on their risk of developing complications or disease progression, enabling personalized treatment strategies.
- Emphasize the importance of lifestyle modifications, such as a balanced diet rich in antioxidants, regular physical activity, and stress management, to mitigate oxidative stress, inflammation, and lipid peroxidation in cardiac patients.

By implementing these recommendations, healthcare professionals can enhance the early detection, risk stratification, and management of cardiovascular diseases, ultimately improving patient outcomes and quality of life.

Contribution to knowledge

1. The study clearly demonstrates significantly higher levels of 8-OHdG, IL-8, and MDA in cardiac patients compared to healthy controls. These biomarkers are indicators of oxidative DNA damage, inflammation, and lipid peroxidation, respectively. The elevated levels observed in cardiac patients provide direct evidence of the involvement of these pathophysiological processes in cardiovascular diseases. This finding corroborates previous research but also adds to the body of knowledge by providing specific data from a Nigerian population.
2. A notable contribution of this study is the identification of strong positive correlations among the levels of 8-OHdG, IL-8, and

MDA in the study participants. The Pearson correlation coefficients indicated a significant positive relationship between each pair of these biomarkers. This suggests that high levels of one biomarker are associated with high levels of the other two, implying potential interconnections or shared underlying mechanisms.

While previous studies have examined these biomarkers individually in relation to cardiovascular diseases, the correlation between all three parameters has not been widely reported. This finding suggests that oxidative stress, inflammation, and lipid peroxidation may be interlinked processes in the pathogenesis of cardiovascular diseases, and the measurement of these biomarkers together could provide a more comprehensive assessment of disease risk or progression.

3. This novel contribution to knowledge highlights the importance of considering these processes collectively, rather than in isolation, when evaluating and managing cardiac patients. The strong correlations observed in this study warrant further investigation into the potential crosstalk or feedback mechanisms among oxidative stress, inflammation, and lipid peroxidation in the context of cardiovascular diseases. This research not only reinforces the involvement of oxidative stress, inflammation, and lipid peroxidation in cardiac diseases but also contributes new knowledge by demonstrating the correlations among these pathways in a Nigerian population. The findings suggest the potential utility of combining these biomarkers for better risk stratification and disease management strategies in cardiac patients.

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Citation: Moronkeji A.I., Olaniyan M.F. Evaluation of 8-Hydroxy-2'-Deoxyguanosine, Interleukin-8 and Malondialdehyde Among Cardiac Patients in Ibadan, Oyo State. *Sokoto Journal of Medical Laboratory Science*; **9(2)**: 160 – 169. <https://dx.doi.org/10.4314/sokjmls.v9i2.19>

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