

Original Research Article

Assessment of oxidative stress biomarkers in steady and crisis states of sickle cell disease

Akinloye Bankole Oyetunde^{1*}, Ayodeji O Olayanju^{1,2}, Adeola O Oluboyo¹, Emmanuel A Omon¹, Chisara S Okolo¹, Yashim A Nuhu³, Ayuba Sunday Buru⁴

¹Department of Medical Laboratory Science, College of Medicine and Health Sciences, Afe Babalola University Ado-Ekiti, Ekiti State, Nigeria, ²Department of Biomolecular Science, Kingston University, London, United Kingdom, ³Department of Medical Laboratory Science, Baze University, Abuja, ⁴Department of Medical Laboratory Science, Faculty of Allied Health Sciences, College of Allied Health and Pharmaceutical Sciences, Kaduna State University, Kaduna, Nigeria

*For correspondence: **Email:** oyetundeab@pg.abuad.edu.ng

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Abstract

Purpose: To assess the oxidative stress parameters in steady and crisis state patients with sickle cell disease (Hb SS) in Nigeria, which has the highest prevalence of the monogenetic disorder.

Methods: Blood specimens were collected from 100 Hb SS participants (comprising 50 in steady state, 50 in crises with > three transfusions in a year) and 50 Hb AA apparently healthy, non-transfused controls with normal blood values between January 2023 to June 2024. Malondialdehyde (MDA), superoxide dismutase (SOD) Catalase levels in plasma were determined using Enzyme-Linked Immunosorbent Assay (ELISA).

Results: Superoxide dismutase (SOD) and catalase were significantly lower ($p < 0.05$), while malondialdehyde was significantly higher in sickle cell subjects compared to control group ($p < 0.05$). Catalase levels were reduced in crises and steady state sickle cell disease (SCD) compared to control group. The SOD was significantly higher in female subjects in both steady state and crisis ($p < 0.05$). There was no significant difference in Catalase (CAT) and Superoxide dismutase (SOD) with respect to gender and age ($p > 0.05$).

Conclusion: There is evidence of an increase in oxidative stress in individuals with SCD. The further reduction in antioxidant capacity during VOC relative to steady state underscores that oxidative damage is significantly aggravated during acute crises. This suggests that excessive generation of reactive oxygen species (ROS) and reduced antioxidant defense mechanisms contribute substantially to the pathophysiology of sickle cell anemia.

Keywords: Sickle cell disease, Oxidative stress, Antioxidant, Lipid peroxidation, Transfusion

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INTRODUCTION

Sickle cell disease (SCD) is a hereditary condition characterized by chronic hemolysis, vaso-occlusion, and recurrent episodes of acute and chronic complications. A mutation in the β -globin gene of individuals with hemoglobin S

(HbS) produces defective hemoglobin which can polymerize in low-oxygen environments, causing blood cells (RBCs) to adopt a sickle shape. Sickled RBCs are more prone to hemolysis, stick more to the endothelium, leading to ischemia-reperfusion injury, oxidative stress, and systemic inflammation [1]. Oxidative stress occurs where

there is an imbalance between pro-oxidants and antioxidants, leading to an overproduction of reactive oxygen species (ROS) [2]. In SCD, oxidative stress is exacerbated by chronic hemolysis, repeated episodes of hypoxia-reoxygenation, and ongoing inflammatory responses. Increased ROS production in patients with SCD during severe anemia, stroke, and acute chest syndrome can cause damage to cells and tissues, worsening complications such as endothelial dysfunction, organ failure, susceptibility to infections, and vaso-occlusive crises (VOC) [3]. Blood transfusion is an important treatment for managing severe anemia, preventing strokes, and reducing complications in patients with SCD. However, recurrent blood transfusions present additional challenges, particularly iron overload and oxidative stress, which can worsen the disease [4]. Individuals with SCD have red blood cells (RBCs) that are more vulnerable to oxidative damage as a result of increased production of reactive oxygen species (ROS) from hemolysis and HbS auto-oxidation. Excess iron from frequent transfusions can trigger ROS formation through the Fenton reaction, leading to heightened oxidative damage to lipids, proteins, and DNA. This contributes to cellular dysfunction and apoptosis [5]. Furthermore, transfused RBCs undergo oxidative changes during storage, and when transfused, they can increase the existing oxidative burden in patients with SCD [6]. However, antioxidant defense mechanisms, including enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as non-enzymatic antioxidants such as glutathione (GSH) and vitamins C and E, are often overwhelmed in individuals with SCD [7].

Despite the increasing use of transfusion therapy in managing SCD, there has been limited investigation into the effects of multiple transfusions on oxidative stress markers in these individuals. Although oxidative stress is recognized as a significant factor in SCD-related complications, there is a lack of data regarding how transfusion-associated oxidative stress affects disease progression, organ damage, and overall patient outcomes. Sickle cell disease is characterized by chronic hemolysis and recurrent vaso-occlusive crises that predispose patients to heightened oxidative stress. Frequent blood transfusions, a mainstay in SCD management, can further exacerbate oxidative imbalance through increased iron load and reactive oxygen species generation.

Evaluating oxidative stress markers in transfused SCD patients across crisis and steady states is

therefore essential for elucidating the biochemical alterations associated with transfusion therapy. Understanding these patterns will provide a scientific basis for developing targeted interventions aimed at mitigating oxidative damage, improving transfusion practices, and enhancing patient outcomes. The findings could inform better patient management approaches, such as optimizing transfusion protocols, incorporating antioxidant therapy, and implementing effective iron chelation strategies, thereby improving clinical outcomes and overall quality of life for individuals living with SCD.

METHODS

Study design and population

This hospital-based cross-sectional study was carried out from January 2023 to June 2024. Recruitment was at sickle cell clinics and haematology wards in Asokoro General Hospital, Maitama District Hospital and Wuse General Hospital in Abuja, Nigeria. A total of 150 participants, comprising 100 sickle cell patients (50 steady state and 50 vaso-occlusive crises), and 50 controls, were included in the study. A structured questionnaire was used to obtain clinical information and demographic characteristics. Haemoglobin electrophoresis was used to confirm phenotype of all participants. Sickle cell disease patients in the study were grouped into two: those with vaso-occlusive crisis (VOC) and steady state. Steady state was clinically defined as patient without acute painful crisis requiring treatment, no history of transfusion in the last 4 months and no hospital admission in the last 3 weeks. Vaso-occlusive crisis means patient on admission with pain in the bones, muscles, and joints not attributable to any other cause after examination by the physician or haematologist.

Sample size calculation

The sample size was determined using Leslie Fisher's formula for estimating the minimum sample size [8]. Using this method, a minimum of 45 patients were required for each group but 50 patients were recruited to increase the precision and statistical power. The standard normal deviation was set at a 95 % confidence level, with the allowable margin of error of 5 %.

Ethics approval and consent to participate

All aspects of this study complied with the Helsinki declaration [9]. Ethical clearance was obtained from the Research Ethical Review

Committee in the Federal Ministry of Health, Abuja (FHREC/2023/01/180/01-09023) and Health Research Ethical Committee of College of Medicine and Health Science, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria (ABUADHREC/6/06/2024/402). Informed consent was obtained from each participant.

Inclusion and exclusion criteria

Inclusion criteria include patients diagnosed with sickle cell anemia (HbSS genotype) through hemoglobin electrophoresis, having received at least three or more blood transfusions in the past year, and having consented to participate in the study. Exclusion criteria include patients with other hemoglobinopathies (HbSC, HbCC), history of liver disease, renal failure, metabolic syndrome, obesity or malignancies, patients on antioxidant supplementation, pregnant women and patients who refused to give consent.

Sample collection

Three milliliters of blood sample were collected from each participant aseptically using the venipuncture technique and dispensed into sterile plain bottle, allowed to clot, then centrifuged at 2000g for 5 min using a bucket centrifuge (Uniscope laboratory centrifuge, Model: SM112, England) in order to obtain clear non-hemolysed serum. The separated serum samples were transferred into sterile labelled plain bottles, stored frozen at -20 °C prior to the time of analysis.

Enzyme-linked immunosorbent assay (ELISA)

Malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and Nitric oxide (NO) were estimated by ELISA kits (Elabscience Biotechnology Inc., USA) in accordance with instructions by the manufacturers.

Statistical analysis

Statistical analysis was done using IBM Statistical Package for the Social Sciences (SPSS) version 25.0 to determine the mean, standard deviation, correlations and one-way analysis of variance (ANOVA) among study groups. $P < 0.05$ was considered as level of significance.

RESULTS

Socio-demographic characteristics of the control, subjects in steady state and crisis are reported in Table 1. From the results obtained, 8 and 4 % of the subjects in steady state and crisis have had 1

- 2 crises, 82 and 84 % have had 3 - 5 crises, while 10 and 12 % have had above 6 crises in steady state and crisis, respectively. A total of 94 % of subjects in crisis have had blood transfusions of which 59.6 and 40.4 % had 3 - 5 transfusions and above 6 transfusions, respectively. About 72.9 and 74.5 % of the subjects in steady state and crisis have experienced transfusion reactions, respectively. The frequency of painful crises in steady state subjects was 1-2 (44 %) and 3 - 5 (56 %), while in subjects in crisis was 38 and 62 %, respectively. Figure 1 shows SOD activity in normal control, subjects in crisis and steady state. From the result obtained, SOD was significantly lower ($p < 0.05$) in sickle cell subjects in crisis and steady state compared to control group, and significantly lower ($p < 0.05$) in SCD subjects in crisis compared to SCD subjects in steady state. Figure 2 shows the activity of MDA and total antioxidant status (TAS) in normal control, SCD subjects in crisis and steady state. Total antioxidant status was significantly lower ($p < 0.05$) in sickle cell subjects in crisis and steady state compared to control group, and significantly lower ($p < 0.05$) in SCD subjects in crisis compared to SCD subjects in steady state, while MDA was significantly higher ($p < 0.05$) in SCD subjects in crisis and steady state compared to control, and significantly higher ($p < 0.05$) in crisis SCD subjects compared to SCD subjects in steady state. Table 2 and Table 3 show the oxidative stress biomarkers of SCD subjects in steady state and crisis according to gender. The results obtained showed that SOD was significantly ($p < 0.05$) higher in female SCD subjects in both steady state and crisis compared to male SCD subjects. There was no significant difference in the other oxidative parameters (MDA and TAS) studied with respect to gender ($p > 0.05$). Table 4 and Table 5 show the oxidative stress biomarkers of SCD subjects in steady state and crisis according to age. The results obtained showed that there was no statistically significant difference in all parameters studied in both steady state and crisis according to age ($p > 0.05$).

DISCUSSION

Sickle cell disease is characterized by chronic hemolysis and recurrent vaso-occlusion that produce sustained generation of reactive oxygen and nitrogen species, depletion of endogenous antioxidant defenses (notably glutathione and enzymatic systems such as superoxide dismutase and catalase), and accumulation of pro-oxidant hemolysis products (free heme/hemin).

Table 1: Clinical details of the sickle cell subject in crisis and steady state (n=50)

Variable	Steady state	Crisis
Frequency of crisis		
1-2	4 (8.0%)	2 (4.0%)
3-5	41 (82.0%)	42 (84.0%)
6 & above	5 (10.0%)	6 (12.0%)
Transfused		
Yes	48 (96.0%)	47 (94.0%)
No	2 (4.0%)	3 (6.0%)
Frequency of transfusion		
1-2	11 (22.9%)	0 (0%)
3-5	26 (54.2%)	28 (59.6%)
6 & above	11 (22.9%)	19 (40.4%)
Experience a transfusion reaction		
Yes	35 (72.9%)	35 (74.5%)
No	13 (27.1%)	12 (25.5%)
Use routine drugs		
Yes	46 (92.0%)	47 (94.0%)
No	4 (8.0%)	3 (6.0%)
Frequency of painful crises		
1-2	22 (44.0%)	19 (38.0%)
3-5	28 (56.0%)	31 (62.0%)
Experienced stroke		
Yes	0 (0%)	0 (0%)
No	50 (100%)	50 (100%)
Experienced priapism		
Yes	0 (0%)	0 (0%)
No	50 (100%)	50 (100%)

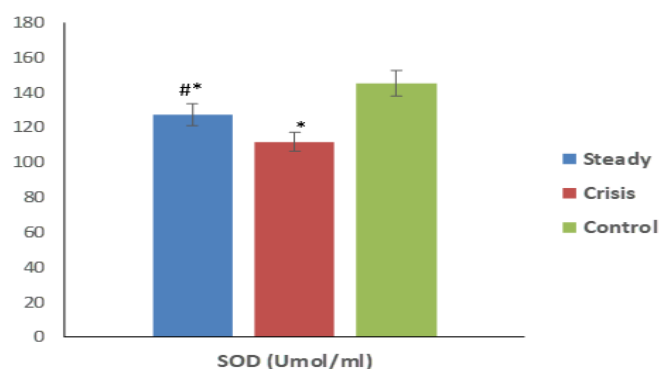
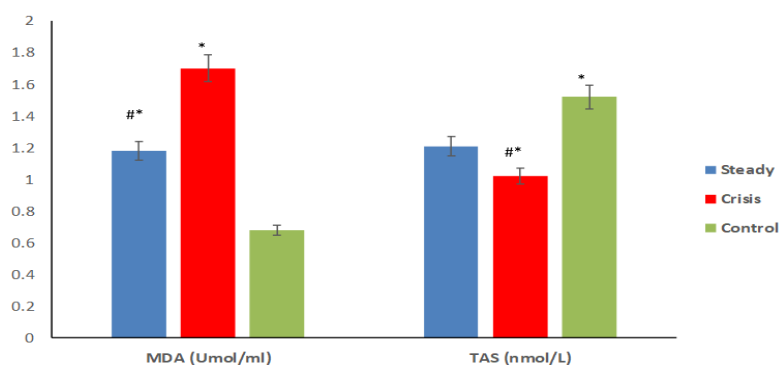
**Figure 1:** Superoxide dismutase (SOD) in normal control, SCD subjects in crisis and steady state. Keys: * $P < 0.05$; Steady state and crisis vs normal control, # $p < 0.05$, Steady state vs crisis**Figure 2:** Malonaldehyde (MDA) and total antioxidant status (TAS) in normal control, subjects in crisis and steady state. Keys: * $P < 0.05$, Steady state and crisis vs normal control; # $p < 0.05$ Steady state vs crisis

Table 2: Oxidative stress biomarkers of SCD subjects with respect to gender in steady state

Variable	Male	Female	P-value
Malonaldehyde (nmol/mL)	0.89±0.26	0.81±0.25	0.236
Superoxide dismutase (Umol/mL)	126.36±7.14	132.47±5.89	0.003*
Catalase (pg/mL)	30.65±8.39	31.69±4.33	0.566

Values are mean ± SD; **P* < 0.05

Table 3: Oxidative stress parameters of SCD subjects in crisis according to gender

Variable	Male	Female	P-value
Malonaldehyde (nmol/mL)	1.09±0.24	0.99±0.52	0.630
Superoxide dismutase (Umol/mL)	111.17±6.20	119.36±5.66	0.002*
Catalase (pg/mL)	25.67±2.44	27.88±3.66	0.168

Values are mean ± SD; **P* < 0.05

Table 4: Oxidative stress biomarkers of SCD subjects in steady state according to age

Variable	1–10 years	11–15 years	16–20 years	P-value
Malonaldehyde (nmol/mL)	0.89±0.61	0.92±0.39	0.88±0.40	0.788
Superoxide dismutase (Umol/mL)	120.26±4.25	122.84±4.32	123.11±4.07	0.505
Catalase (pg/mL)	29.90±4.41	30.21±4.63	29.64±5.10	0.399

Values are mean ± SD

Table 5: Oxidative stress biomarkers of SCD subjects in crisis according to age

Variable	1–10 years	11–15 years	16–20 years	P-value
Malonaldehyde (nmol/mL)	1.10±0.26	1.13±0.25	1.16±0.56	0.398
Superoxide dismutase (Umol/mL)	115.51±6.17	117.65±6.77	114.98±6.74	0.728
Catalase (pg/mL)	24.36±4.28	25.69±4.40	26.26±5.01	0.619

Values are mean ± SD

These redox disturbances mechanistically amplify endothelial activation, inflammation and red cell membrane injury key drivers of vaso-occlusive crises and progressive organ dysfunction. Empirical studies and early clinical trials show that targeted antioxidant strategies can restore redox balance and reduce biochemical markers of oxidative injury; however, definitive evidence that antioxidant therapy reduces clinically important outcomes is lacking. Therefore, investigating antioxidant interventions as adjunctive therapies is scientifically justified: mechanistic plausibility is strong, preliminary biomarker and small-trial data are encouraging, and there is an unmet need for complementary approaches that address pathogenic pathways not fully corrected by currently available disease-modifying treatments. Large, randomized trials with clinical endpoints are required to confirm efficacy, optimize dosing and establish safety. Superoxide dismutase (SOD) is an essential enzyme that converts superoxide radicals into hydrogen peroxide, playing a significant role in antioxidant defense, particularly in cells involved in aerobic metabolism [10].

There was a significantly lower mean serum SOD level in individuals with HbSS compared to those with HbAA, with even lower levels observed in HbSS patients during a crisis. While previous studies have consistently reported

reduced superoxide dismutase (SOD) activity in patients with sickle cell anemia (SCA) compared to HbAA controls, and attributed this to factors such as zinc deficiency, the present study differs in several key aspects. Unlike earlier research, this study specifically evaluates SOD activity in SCD patients who have undergone multiple blood transfusions, assessing variations between crisis and steady states. By integrating transfusion history and clinical state into the analysis, it provides a more nuanced understanding of how oxidative stress and antioxidant enzyme activity fluctuate with transfusion exposure and disease severity. This focus on the combined impact of transfusion therapy and oxidative imbalance fills a critical knowledge gap left by previous investigations that largely considered SOD activity in untreated or non-transfused patients [10,11]. One possible explanation for this disparity is zinc deficiency, which has been noted in SCA patients [8]. Zinc deficiency can decrease SOD activity, as the enzyme requires zinc as a cofactor for optimal function. However, the variation in enzyme activity between SCD patients and HbAA controls may also be due to increased demand and rapid degradation of SOD caused by elevated levels of reactive oxygen species (ROS) [12].

During a vaso-occlusive crisis, excessive production of reactive oxygen species (ROS) overwhelms the antioxidant defense system, leading to oxidative damage of lipids, proteins, and cell membranes. Superoxide dismutase (SOD), a key antioxidant enzyme that neutralizes superoxide radicals, becomes depleted under such stress. Zinc plays a crucial structural and catalytic role in the activity of SOD (particularly Cu/Zn-SOD); hence, zinc deficiency compromises its function and reduces the body's capacity to detoxify ROS. In this study, the interaction between zinc deficiency and oxidative stress was explored in sickle cell disease (SCD) patients, comparing those in steady state and vaso-occlusive crisis to healthy HbAA controls. Because HbS auto-oxidizes more rapidly than HbA, SCD patients inherently generate higher levels of ROS, predisposing them to oxidative stress. This condition is exacerbated during crises when hemolysis and ischemia–reperfusion cycles amplify ROS production, further depleting zinc-dependent enzymes.

Frequent blood transfusions, a common therapeutic intervention in SCD, may influence oxidative balance in two ways: by temporarily improving oxygen delivery and reducing sickling, yet also by introducing excess free iron that catalyzes ROS formation via the Fenton reaction. This dual effect complicates oxidative homeostasis and may modulate antioxidant enzyme activity depending on the patient's transfusion frequency and post-transfusion iron chelation status. Therefore, the study's comparative analysis of zinc levels and SOD activity in transfused and non-transfused SCD patients provides valuable insight into how micronutrient deficiencies and oxidative stress interact within different treatment contexts. Understanding this relationship may help refine transfusion protocols and guide antioxidant or zinc supplementation strategies to improve clinical outcomes in SCD management [13]. Low SOD levels may indicate a need for antioxidant therapy, as this enzyme plays a crucial role in protecting against oxidative stress [14].

Several studies document that SCD patients have significantly reduced SOD (and CAT) enzyme activities compared to healthy controls (for example, erythrocyte SOD and CAT were significantly lower in Ghanaian SCD patients vs HbAA controls). While this indicates an impaired endogenous antioxidant defence in SCD and provides a rationale for antioxidant-based interventions, there is currently no robust clinical trial evidence that direct supplementation with SOD (or SOD-mimetics) improves outcomes in SCD. Indeed, a recent systematic review found

inconsistent effects of antioxidant nutrient supplements and calls for more high-quality trials. In this study, catalase (CAT) was found to be significantly lower in individuals with SCD in both crisis and steady state compared to controls. (E.g., in the Ghana study, CAT levels were significantly lower in HbSS vaso-occlusive crisis patients vs controls). SCD patients show significantly reduced SOD activity compared to healthy controls, and this reduction is worse during vaso-occlusive crisis. For example, one study found serum SOD in HbSS patients during crisis at 1.755 U/mL, steady state at 1.844 U/mL, versus controls 1.928 U/mL ($p = 0.001$). Genetic studies show polymorphisms in the SOD2 (mitochondrial Mn-SOD) genes (e.g., Val16Ala) are associated with lower SOD2 levels and worse disease severity in SCD. Preclinical work: In vessels from SCD-model mice, treatment with an SOD mimetic (MnTE-2-PyP) significantly restored acetylcholine-dependent relaxation, suggesting functional vascular benefits of SOD-mimetics in SCD context. Reviews of antioxidant supplementation in SCD indicate that although the oxidative stress paradigm is strong, clinical evidence for benefit of antioxidant therapies is inconsistent and limited. For example, the recent Cochrane review: “antioxidant supplementation for sickle cell disease” notes conflicting evidence and no consensus for routine use.

No randomized controlled trial in SCD patients specifically evaluates exogenous SOD supplementation (i.e., giving SOD enzyme or SOD-mimetic drug) and outcomes (e.g., reduction in crisis rate, hemolysis, oxidative damage) are robustly demonstrated. While SOD-mimetics have been developed (for other conditions) and mechanistic data exist, translation into SCD for human treatment remains investigational. For example, SOD mimetics: “MnSOD mimetics offer innovative strategies but future research should optimise stability, bioavailability, and safety. The human antioxidant supplementation trials in SCD (vitamins, trace elements) generally do not target SOD enzyme supplementation per se, and often have mixed outcomes (e.g., high-dose vitamin C/E, etc) with unclear benefit. Many studies demonstrate that patients with SCD have significantly lower SOD enzyme activity compared to healthy controls. For example, Uche *et al* reported mean serum SOD in HbSS crisis at 1.755 U/mL versus 1.928 U/mL in controls; $p = 0.001$ [10]. This under-functioning antioxidant system provides a rationale for therapeutic antioxidant strategies such as SOD-mimetics. However, to date, no large-scale randomized clinical trial has definitively shown

that direct SOD supplementation improves clinical outcomes in SCD; thus, these interventions remain investigational. While previous studies [15-16] reported decreased catalase levels in SCD patients, the present study differs by evaluating CAT activity across both steady-state and vaso-occlusive crisis conditions in patients who have undergone multiple blood transfusions. Additionally, this study examines the interaction between CAT activity and other antioxidant markers, providing a more comprehensive assessment of oxidative stress dynamics in SCD patients [15-16].

The body's protective mechanism to scavenge reactive oxygen species (ROS), especially hydrogen peroxide (H_2O_2), may lead to an increase in CAT activity, or it could be a result of the higher reticulocyte content in the blood of SCD patients. Conversely, chronic and overwhelming oxidative stress may have caused the decrease in CAT activity [17]. On the other hand, some studies observed increased CAT levels in SCD subjects [18]. An increase in CAT may have a preventive effect in scavenging peroxides, while decreased levels could be attributed to severe and prolonged oxidative stress. The imbalance between overproduction of free radicals and the inability of antioxidants within cells to eliminate them will lead to oxidative stress and inflammation in individuals with SCD [14]. CAT is expressed in all major human body organs and red blood cells (RBCs), playing a crucial role in cellular defense against oxidative stress. However, catalase has limited therapeutic uses due to its lower intracellular delivery, quick elimination, short half-life, poor operational stability, and limited reusability as an enzyme [15]. The lower levels of erythrocyte SOD and CAT in SCD subjects may be attributed to the severity of oxidative stress [15]. Haemoglobin S, present in the erythrocytes of SCD patients, oxidizes faster compared to normal haemoglobin, resulting in increased ROS production, leading to lipid peroxidation and potential inactivation of antioxidant enzymes [13].

Superoxide dismutase and CAT may become overwhelmed after ischemia and reperfusion, leading to elevated ROS levels in these patients [19]. Levels of antioxidant enzymes may be influenced by various factors, including the clinical condition of patients, genotype, and chronic hemolysis. The results of the study indicate that acute complications in SCD patients play a significant role in their oxidative stress status. HbSS patients experiencing vaso-occlusive crisis (VOC) exhibited significantly lower levels of antioxidant enzymes compared to those in steady state. The excessive ROS

production during VOC may have contributed to the decreased levels of SOD and CAT in these patients. Additionally, differences in SOD and CAT levels among SCD patients in crisis and steady state are partly due to the intensity of hemolysis and rapid auto-oxidation of the hemoglobin S molecule in individuals with the HbSS genotype [15]. The study findings demonstrate that patients with the HbSS genotype exhibit significantly reduced levels of superoxide dismutase (SOD) and catalase (CAT), reflecting substantial depletion of key antioxidants, including nitric oxide and essential vitamins. Malondialdehyde (MDA), a well-established biomarker of lipid peroxidation, was significantly elevated in individuals with sickle cell disease (SCD) during both steady state and vaso-occlusive crisis (VOC) compared to controls [14,15,20,21]. The increase in MDA is directly related to enhanced reactive oxygen species (ROS) generation, which intensifies oxidative damage, particularly during VOC.

Variations in MDA levels among HbSS patients correspond with the degree of hemolysis and underlying chronic conditions, indicating that the sickle cell genotype directly influences lipid peroxidation [15]. MDA accumulation disrupts the organization of phospholipids in erythrocyte membranes, promoting oxidation of plasma and organelle membranes, including mitochondria, thereby impairing cellular function [21]. Membrane damage contributes to the formation of irreversible sickle cells, a key pathogenic feature in SCD [3]. Furthermore, elevated MDA levels result from increased ROS production and iron auto-oxidation, which alter protein structures, modify amino acid side chains, and compromise the activity of antioxidant enzymes and relevant protein receptors [15,22]. Elevated serum MDA has been associated with multiple systemic disorders, including diabetes mellitus, various cancers, premalignant lesions, leukoplakia, oral submucous fibrosis, and SCD [23]. The study further revealed that oxidative stress markers and SOD levels were higher in female HbSS subjects during both crisis and steady states. This difference reflects estrogen-mediated antioxidant protection, which has been shown to modulate nitric oxide synthase activity, improve lipid profiles, and enhance endothelium-dependent vasodilation [24,25]. The altered oxidative profile in male HbSS patients aligns with reports of a higher incidence of VOC and SCD-related complications in males compared to females [26–27]. SOD levels were significantly higher ($p < 0.05$) in female SCD subjects, whereas other oxidative parameters did not differ significantly with respect to gender or age ($p > 0.05$).

Total Antioxidant Status (TAS), which represents a dynamic equilibrium of serum antioxidants rather than a simple sum of individual components, was significantly lower in SCD subjects during both crisis and steady state compared to controls [20]. This finding highlights the compromised antioxidant defense system in SCD, emphasizing the need for interventions aimed at restoring redox balance. Considering that study participants had received 2–3 transfusions, these results also reflect the cumulative oxidative impact of transfusion therapy on antioxidant depletion and ROS generation. Additionally, TAS was significantly lower in subjects in crisis compared to those in steady state. The decreased TAS levels found in this study align with previous research that reported lower values of nonenzymatic or enzymatic antioxidants in individuals with Sickle Cell Disease (SCD) during vaso-occlusive crisis compared to those in steady state [15,28]. The decline in antioxidant status levels during vaso-occlusive crisis can be attributed to increased consumption of antioxidants to combat elevated levels of free radicals and reactive oxygen species (ROS) generated during the crisis [28]. Individuals with SCD are more prone to inflammation than those without SCD [29].

The higher mean serum TAS levels observed in SCD subjects in steady state compared to those in crisis may represent an adaptive mechanism to counteract oxidative stress in chronic inflammatory conditions like SCD. Conversely, the decreased mean serum TAS levels in SCD during vaso-occlusive crisis may be due to the overwhelming of the body's antioxidant properties fighting the oxidative stress associated with SCD. Previous studies have shown inconsistencies in the levels of enzymatic antioxidants such as catalase in SCD subjects [29], which could also impact TAS levels as seen in this study. Patients with the lowest TAS levels tend to experience a higher number of vaso-occlusive episodes [20], suggesting that depletion in low molecular weight antioxidants may contribute to vaso-occlusive crises.

CONCLUSION

Antioxidant activity is lower in sickle cell disease patients compared to controls and is even lower during vaso-occlusive crisis when compared with sickle cell disease patients in steady state. This confirms the increased oxidant activity in sickle cell anemia which is worsened during crises. These findings suggest that patients with SCD have increased lipid peroxidation in addition to an imbalance in their pro-oxidant and antioxidant states.

DECLARATIONS

Acknowledgement/Funding

None.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Ethics approval

Ethical clearance was obtained from the Research Ethical Review Committee in the Federal Ministry of Health, Abuja (FHREC/2023/01/180/01-09023) and Health Research Ethical Committee of College of Medicine and Health Science, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria (ABUADHREC/6/06/2024/402).

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. AB Oyetunde wrote the first draft and coordinated the research. The protocol was designed by AO Olayanju and CS Okolo to facilitate sample collection. EA Omon and YA Nuhu carried out statistical analysis. AO Olayanju and AS Buru supervised the work.

REFERENCES

1. Inusa BPD, Hsu LL, Kohli N, Patel A, Ominu-Evbota K, Anie KA, Atoyebi W. Sickle cell disease-genetics,

- pathophysiology, clinical presentation and treatment. *Int J Neonatal Screen* 2019; 5(2): 20.
2. Tebbi CK. Sick cell disease: A review. *Hemato* 2022; 3(2): 341-366.
 3. Vona R, Sposi NM, Mattia L, Gambardella L, Straface E, Pietraforte D. Sick cell disease: role of oxidative stress and antioxidant therapy. *Antioxidants* 2021; 10(2): 296.
 4. Tanhehco YC, Shi PA, Schwartz J. Transfusion therapy in sickle cell disease. *Ann Blood* 2022; 7: 9.
 5. Wang Q, Zennadi R. The role of RBC oxidative stress in sickle cell disease: From the molecular basis to pathologic implications. *Antioxidants (Basel)* 2021; 10(10): 1608.
 6. Pavitra E, Acharya RK, Gupta VK, Verma HK, Kang H, Lee H, Sahu T, Raju GR, Huh YS. Impacts of oxidative stress and anti-oxidants on the development, pathogenesis, and therapy of sickle cell disease: A comprehensive review. *Biomed Pharmacother* 2024; 176: 116849
 7. Jomova K, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Arch Toxicol* 2024; 98(5):1323-1367.
 8. Yamane T. *Statistics, An introductory analysis*, 2nd Ed., 1967. New York: Harper and Row.
 9. World Medical Association. *World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects*. *JAMA* 2013; 310(20): 2191-2194.
 10. Uche E, Olowoselu F, Augustine B, Suleiman A, Ismail A, Oluwole E. Superoxide dismutase activity in sickle cell anemia patients during crisis and in steady State. *J Applied Hematol* 2020; 11(1): 59-63.
 11. Ren H, Ghebremeskel K, Okpala I, Lee A, Ibegbulam O, Crawford M. Patients with sickle cell disease have reduced blood antioxidant protection. *Int J Vitamin Nutrition Res* 2018; 78(2): 139-147.
 12. Okocha C, Manafa P, Aneke J, Onwuzurike C, Ibeh N, Chukwuma O. Serum superoxide dismutase activity: A predictor of disease severity in Nigerian sickle cell anemia patients in steady state. *Medical J DY Patil University* 2017; 10(2): 406-411.
 13. Oyeyemi A, Oyebanji G, Akinlua I. Evaluation of antioxidant enzymes in children with sickle cell anemia in Ekiti state, Nigeria. *J Med Dental Sci Res* 2016; 3(1): 1-4.
 14. Shanan WF, Thuwaini MM, Khudhair HA. Evaluation of the possible pathophysiological role of redox status in patients with sickle cell anemia. *J Applied Hematol* 2024; 15(3): 219-227.
 15. Antwi-Boasiako C, Dankwah GB, Aryee R, Hayfron-Benjamin C, Donkor ES, Campbell AD. Oxidative profile of patients with sickle cell disease. *Med Sci* 2019; 7(2): 17-24.
 16. Engwa GA, Okolie A, Chidili JPC, Okore PA, Onu PC, Ugwu MO. Relationship of oxidative stress and antioxidant response with vaso-occlusive crisis in sickle cell anaemia. *African Health Sci* 2021; 21(1): 150-158.
 17. Chirico EN, Pialoux V. Role of oxidative stress in the pathogenesis of sickle cell disease. *IUBMB Life* 2022; 64(2): 72-80
 18. Hadi ZA, Fadhil JA, Atheer HO, Hawra A. Serum antioxidant status in sickle cell disease patients: implications for oxidative stress and disease severity. *J Contemporary Medical Sci* 2023; 9(2): 106-110
 19. Bernard KFC, Cabral BNP, Bernard C, Flora NL, Anatole PC, Donatien G. Electrolytic and oxidative stress profile of sickle cell anaemia patients in Cameroon: The effect of some extrinsic factors. *Asian Hematol Res J* 2018; 1(1): 1-11.
 20. Okorie CP, Nwagha T, Ejezie F. Assessment of some indicators of oxidative stress in Nigerian sickle cell anemic patients. *Annals Afri Med* 2020; 17(3): 116-125.
 21. Omon EA, Ajayi OD. Oxidative stress and antioxidant markers in individuals with thyroid hormone dysfunction. *Eur J Clin Exper Med* 2024; 21(4): 768-775
 22. Erhabor O, Jiya NM, Abubakar MB, Usman S. Malondialdehyde and haptoglobin among sickle cell disease children in Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. *Paediatrics* 2020; 11(4): 105-116.
 23. Baliga S, Chaudhary M, Bhat S, Bhansali P, Agrawal A, Gundawar S. Estimation of malondialdehyde levels in serum and saliva of children affected with sickle cell anemia. *J Ind Soc Prevent Med* 2018; 36(1): 43-47.
 24. Ama-Moor VJ, Pieme CA, Chemegne BC, Manonji H, Njinkio-Nono BL, Mamiafo CT, Moukette BM, Nzufu FT, Tazoacha A. Oxidative profile of sickle cell patients in a Cameroonian urban hospital. *BMC Clin Pathol* 2019; 16(2): 15-20.
 25. Biswal S, Rizwan H, Pal S, Sabnam S, Parida P, Pal A. Oxidative stress, antioxidant capacity, biomolecule damage, and inflammation symptoms of sickle cell disease in children. *Hematol* 2019; 24(1): 1-9.
 26. Antwi-Boasiako C, Dankwah GB, Aryee R, Hayfron-Benjamin C, Aboagye G, Campbell AD. Correlation of lipid peroxidation and nitric oxide metabolites, trace elements, and antioxidant enzymes in patients with sickle cell disease. *J Clin Lab Anal* 2020; 34(1): 23294-23299
 27. Mao C, Yuan JQ, Liv YB, Gao X, Yin ZX, Kraus VB. Associations between superoxide dismutase, malondialdehyde and all-cause mortality in older adults: A community-based cohort study. *BMC Geriatrics* 2019; 19(1): 104-113.
 28. Uju CM, Ulasi TO, Ezeudu CE, Okpara HC, Elo-Ilo JC, Ezenyeaku CA. Evaluation of total antioxidant status among pediatric sickle cell anemia patients and hemoglobin AA controls. *J Nig Acad Med* 2023; 2(1): 65-70.
 29. Stuart MJ, Nagel RL. Sick cell disease. *Lancet* 2014; 364: 1343-1360. doi: 10.1016/S0140-6736(04)17192-4.