Assessment of Some Indicators of Oxidative Stress in Nigerian Sickle Cell Anemic Patients

C. P. Okorie, Theresa Nwagha¹, Fidelis Ejezie
Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Nigeria Enugu Campus, ¹Department of Hematology and Immunology, University of Nigeria Teaching Hospital, Enugu, Nigeria

Abstract

Background: Oxidative stress, the imbalance between the productive of reactive species and antioxidant defences notably plays an important role in the pathogenesis of sickle cell disease. Generating the necessary data about oxidative stress in patients with sickle cell anemia (SCA) would help in developing cost-effective and accessible treatment interventions of SCA in resource-poor countries. Aim: The aim of this study is to evaluate the levels plasma lipid peroxidation product, malondialdehyde (MDA), serum nitric oxide (NO), and total antioxidant activity (TAOA) as indicators of oxidative stress in sickle cell individuals. Materials and Methods: In this pilot study, 52 patients; homozygous (Hb SS) sickle cell patients confirmed by cellulose electrophoresis and 20 age- and sex-matched healthy (Hb AA) controls were subjected to analysis of NO, MDA, and TAOA. The plasma MDA was measured by a thiobarbituric reaction, (NO) was evaluated by the method described by Guevara et al. using Griess reagent and the TAOA of serum was determined by the method of Koracevic et al. Results: The results showed a statistically significant decrease and increase in baseline levels of NO and MDA, respectively, in Hb SS group when compared with the control Hb AA (P = 0.000 and 95% confidence interval [CI] of 0.10–0.18 and −9.67–−5.57, respectively). A slight decrease in the TAOA level between the groups was observed although not statistically significant (P = 0.15 95% CI was −0.28–2.90). Conclusion: The results showed of imbalance between oxidant and antioxidant status in patients with SCA. Antioxidant supplementation may be a cheap assessable intervention for in sickle cell individuals (in the steady state or in crisis) to prevent further oxidative damage to the erythrocytes.

Keywords: Antioxidants, oxidative stress, sickle cell anemia

Résumé

Contexte: Le déséquilibre entre la production d’espèces réactives et les défenses antioxydantes (stress oxydatif) joue notamment un rôle important rôle dans la pathogénèse de la drépanocytose. Générer les données nécessaires sur le stress oxydatif chez les patients atteints d’anémie falciforme (SCA) aiderait à développer des interventions de traitement rentables et accessibles de SCA dans les pays pauvres en ressources. But: Le but de cette étude est évaluer les taux de peroxydation lipidique plasmatique, de malondialdéhyde (MDA), d’oxyde nitrique sérique (NO) et d’activité antioxydante totale (TAOA) comme indicateurs du stress oxydatif chez les drépanocytaires. Matériels et méthodes: Dans cette étude pilote, 52 patients; homozygote (Hb SS) drépanocytaires conMis via électrophorèse sur cellulose et 20 contrôles sains (Hb AA) appariés selon l’âge et le sexe ont été analysés de NO, MDA et TAOA. La MDA plasmatique a été mesurée par une réaction thiobarbiturique, (NO) a été évaluée par la méthode décrite par Guevara et al. en utilisant le réactif de Griess et le TAOA du sérum a été déterminé par la méthode de Koracevic et al. Résultats: Les résultats ont montré une diminution et une augmentation statistiquement signifi cantes des concentrations initiales de NO et de MDA, respectivement, dans le groupe Hb SS par rapport à contrôle Hb AA (P = 0,000 et 95% intervalle de confiance [IC] de 0,10–0,18 et −9,67––5,57, respectivement). Une légère baisse du niveau TAOA entre les groupes a été observée mais pas statistiquement significative (P = 0,15 IC était de -0,28–2,90). Conclusion: Les résultats ont montré de déséquilibre entre l’état oxydant et antioxydant chez les patients avec SCA. La supplémentation en antioxydants peut être une intervention évaluable bon marché chez les individus atteints de drépanocytose (à l’état stable ou en crise) pour prévenir d’autres dommages oxydatifs aux érythrocytes.

Mots-clés: Antioxydants, stress oxydatif, drépanocytose

Address for correspondence: Dr. Theresa Nwagha, Department of Hematology and Immunology, University of Nigeria Teaching Hospital, Enugu, Nigeria. E-mail: theresa.nwagha@unn.edu.ng

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INTRODUCTION

Sickle cell Anemia a major cause of morbidity and mortality in Africa where there is no readily effective treatment.[1] The disease amounts for over 60% of the world’s major hemoglobinopathies with an estimated 2–3 million Nigerians affected by the S gene.[2] A recent WHO report estimated that around 2% of newborns in Nigeria were affected by sickle cell anemia, giving a total of 150,000 affected children born every year in Nigeria alone.[3] The extent of the problems of sickle cell disease in Nigeria cannot, therefore, be overemphasized because carrier status for the S gene is said to be between 25% and 30%.[1] Furthermore, this is widely acceptable cure for patients with sickle cell anemia though not readily available. Curable methods such as gene therapy and bone marrow transplantation, which may be associated with several complications, are not readily available in developing nations.[1] There is a growing need for cheaply accessible treatment protocol for sickle cell anemia (SCA) patients in resource-poor countries.

Sickle cell disease patients are susceptible to increased oxidative stress.[4-8] Although free radicals are formed by a wide range of normal biochemical processes, they are potentially harmful, and several host defense mechanisms are in place to neutralize their effects.[5] Several defense mechanisms include antioxidant enzymes such as superoxide dismutase (Cu/ZnSOD), catalase, glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase; the nonenzymatic ones which include ascorbic acid (Vitamin C), ubiquinols, glutathione, β-carotene, tocopherols (Vitamin E), albumin, uric acid, bilirubin, and flavonoids which are considered to be the primary defensive system of the cell and some minerals which include copper, iron, zinc, selenium, etc.[5]

Oxidative stress results from the imbalance between the enhanced generation of reactive oxygen species (ROS) and low cellular content of antioxidants.[7] Oxidative stress may play a major role in the pathogenesis of sickle cell anemia by enhancing the sickling phenomenon. Various studies have shown an enhanced production of the ROS and a decreased antioxidant status in SCA.[7] Nitric Oxide (NO) is an important regulator of vascular tone, blood flow, and adhesion. Its bioavailability has been found to decrease in SCD resulting in endothelial dysfunction and vasoconstriction. The sickle erythrocytes generate approximately two times more amounts than usual of superoxide, peroxide, and hydroxyl radicals.[8] These ROS can react with NO converting it into more potent reactive NO species which may damage the cell membrane. This may exaggerate the sickling and hemolytic consequences in sickle cell anemia further; hemolysis may derange NO metabolism that reduces the bioavailability of NO leading to poor vasodilation and vaso-occlusive process.[9]

Lipid peroxidation is usually assessed in humans by measuring malondialdehyde (MDA) which is one of the most popular and reliable markers that determine oxidative stress in clinical situations.[10] An elevated level of MDA has been also observed in previous studies suggesting the excessive formation of ROS by sickle cells.[8,11,12]

With the above view, the present study was aimed to measure the levels of serum NO, total antioxidant activity (TAOA), and plasma MDA as indicators of oxidative stress in sickle cell anemic individuals with a view to recommending antioxidant supplement as a cheap assessable treatment protocol to ameliorate the vaso-oclusive effects of sickle cell disease.

MATERIALS AND METHODS

This present study examined 52 patients; 32 (16 males and 16 females) homozygous (HbSS) sickle cell patients confirmed by cellulose hemoglobin (Hb) electrophoresis attending Haematology Clinic in the University of Nigeria Teaching Hospital, Enugu and 20 age- and sex-matched apparently healthy controls (HbAA). None had a history of concomitant illness such as inflammatory disease; Systemic lupus erythromatosis (SLE) rheumatic heart disease/diabetes mellitus/hypertension or others. Homozygous patients in acute painful crisis, history of blood transfusion within the past 3 months blood transfusion, treatment with hydroxyurea, use of vitamins and trace elements supplementation and pregnancy were excluded from the study. All the cases and controls chosen for the study gave their written consent for participating in the study after being explained the nature of the study. Ethical clearance from the ethics committee of the hospital was obtained.

Hemolized samples were excluded from the analysis. Blood samples were collected in EDTA containers for analysis of MDA, serum NO, and the TAOA of serum. The plasma lipid peroxide product MDA was measured by a thiobarbituric reaction described by Satoh.[13] Serum NO was evaluated by the method described by Guevara et al., using Griess reagent. [14] The TAOA of serum was determined by the method of Koracevic et al.[15] The data analysis was performed using the IBM Statistical Package for Social Sciences statistical software, (SPSS) version 16.0 (Chicago, Illinois). The Student’s t-test was applied to calculate the level of significance at P < 0.05.

RESULTS

A total of 52 patients were studied; 32 with homozygous SS and 20 with homozygous AA as controls. Overall, there were more males than females 30 (n = 52, 58%), 22 (n = 52, 42%), respectively, the mean age in years of test and control subjects were 26 (4.1) and 26 (4.7), respectively. The mean Hb levels for test and control subjects were 7.1 (1.0) and 13.6 (1.4), respectively.

Nitric oxide

The overall mean baseline level of NO of HbSS cases and HbAA control group were 0.13 (0.01) μmol/l and 0.27 (0.01) μmol/l, respectively. The mean NO level for HbSS and Hb AA males are 0.12 (0.05) μmol/l...
and while the mean NO levels for HbSS and HbAA females are 0.13 (0.08) μmol/l and 0.27 (0.04) μmol/l, respectively [Figures 1 and 2]. ANOVA analysis showed the statistically significant difference with a P = 0.000 and F = 35.2. Post hoc analysis showed this difference with P = 0.000 and 95% confidence interval (CI) (0.10–0.19) was observed between the two groups [Tables 1 and 2].

**Malondialdehyde levels**
The mean baseline level of MDA of HbSS patients (test group) and the HbAA patients (control group) were 9.46 (0.63) nmol/ml and 1.84 (0.63) nmol/ml, respectively. The mean MDA level for HbSS and Hb AA males are 9.9 (4.1) nmol/ml and 1.42 (1.2) nmol/ml, respectively, whereas the mean MDA levels for HbSS and HbAA females are 8.8 (2.7) nmol/ml and 1.07 (0.58) nmol/ml, respectively [Figures 3 and 4]. ANOVA analysis showed the statistically significant difference with a P = 0.000 and F value of 40.6. A statistically significant difference with P = 0.000 and 95% CI (−9.6−−5.7) was observed between the two groups [Tables 2 and 3].

**Antioxidant activity levels**
The mean baseline level of TAOA of HbSS patients (test group) and that of the HbAA patients (control group) were 1.48 (0.06) mmol/l and 2.53 (0.82) mmol/l, respectively. The mean AOA level for HbSS and Hb AA males are 1.39 (0.42) mmol/l and 1.67 (0.24) mmol/l, respectively, Figure 4 and 5 whereas the mean AOA levels for HbSS and HbAA females are 1.60 (0.20) mmol/l and 1.78 (0.16) mmol/l, respectively. ANOVA analysis showed the statistically significant difference with a P = 0.150 and F = 1.95. A slight decrease in the level of TAOA of the HbSS group compared to that of the control was observed although not statistically significant (P > 0.05) 95% CI (−0.28–2.40) [Tables 1 and 2].

**DISCUSSION**
The present study has found a significant decrease in the baseline level of serum NO in the Homozygous (HbSS) subjects as compared to the controls [Figure 1]. This is in agreement with several works reported by Emokpae et al., 2010; Arinola et al., 2008; Foluke et al., 2008; Hasanato, 2006, indicating that SCA patients have lower levels of NO, and total antioxidant capacity (TAC) as compared to normal healthy controls.

Among endothelial mediators, NO regulates the normal vascular tone, cellular adhesion, platelet aggregation, and thrombosis. Various studies have demonstrated a state of resistance to the vasodilation due to eNOS mediated impaired blood flow. Recently, it has been shown that this state of NO resistance to the endogenous and exogenous NO, correlated with increased plasma hemoglobin levels which correlate with hemolytic rate and oxidative stress. After hemolysis, hemoglobin is compartmentalized and released into plasma, where it rapidly reacts with NO which destroys it. This results in the abnormally increased consumption of NO forming NO free radicals and ultimately inhibiting vasodilation. The simultaneous release of erythrocyte arginase during hemolysis may limit the availability of arginine to NOS, contributing to a deficiency of NO in the vascular system.

In the present study, the plasma MDA level is significantly elevated, and this agrees with the previous finding. Accumulation of MDA distorts the organization of phospholipids in the human erythrocyte membrane bilayer. The oxidation of phospholipids in the plasma and internal organelle membranes (e.g., the mitochondria) damage their function.

Membrane damage is considered as an important factor contributing towards pathophysiology due to the formation of irreversible sickle cells. Peroxidative reactions have
long been recognized as potential factors that contribute to degenerative cellular processes. Red blood cells (RBCs) are particularly susceptible to peroxidative damage because they contain hemoglobin, one of the most powerful catalysts for initiation of the peroxidative reaction.\[^{25}\] It is also stated that excess quantity of MDA can promote erythrophagocytosis by macrophages.\[^{26}\] The HbS RBC membranes were exposed to increased amounts of the endogenous oxidant. Hb-free iron acts as Fenton’s reagent and produces superoxide, peroxide and hydroxyl radicals, which may further initiate membrane lipid peroxidation.\[^{27}\]

Superoxide/peroxide driven hydroxyl radical (OH) generation is facilitated by membrane-bound hemichrome (HC), a denatured ferric Hb which is found in excessive amounts, bound to the HbS RBC membranes.\[^{28}\] This enhanced oxidative stress may be a contributing factor in the pathogenesis of sickle cell anemia.

Hebbel \[^{8}\] et al. have shown that under ambient oxygen tensions, sickle cells spontaneously generate oxygen radicals (O\[^2\]•−), hydrogen peroxides (H\(_2\)O\(_2\)), and Hydroxyl radicals (OH) approximately two times more when compared to normal RBCs. Furthermore, these workers have also demonstrated

| Table 3: Post hoc tests of ANOVA analysis |
|-----------------------------------------|

| Dependent variable  | Groups                          | Mean difference | SE         | Significant | 95% CI       |
|---------------------|---------------------------------|-----------------|------------|-------------|--------------|
| Nitric oxide levels | Control (test subjects)         | 0.14451*        | 0.01729    | 0.000       | 0.1031       | 0.1859       |
| Malondialdehyde     | Control (test subjects)         | 7.62158*        | 0.85428    | 0.000       | 9.6692       | 5.5740       |
| Total antioxidant activity | Control (test subjects)         | 1.05572        | 0.56018    | 0.151       | 0.2870       | 2.3984       |

*Mean significant difference at \(P<0.05\). SE=Standard error, CI=Confidence interval, HSD=Honest significant difference
that HC may facilitate OH production in the presence of O$_2^-$ and H$_2$O$_2$.

In addition, a slight decrease in the baseline level of serum TAOA of the SCA patients compared to the control group was observed although not statistically significant ($P > 0.05$) [Figure 3] which disagrees with previous studies by Arinola et al., 2008[17] and Foluke et al., 2008.[18] The slight decrease in the level of TAOA observed in these patients could be as a result of apparent healthy living which includes good nutrition. The total antioxidative serum capacity is not a simple sum of the activities of the various antioxidative substances. It is a dynamic equilibrium that is influenced by the interactions between each serum antioxidative constituent.[13] It is thought that the cooperation of antioxidants in human serum provides greater protection against attacks by free radicals than any antioxidant alone. TAC values are more informative than the knowledge of individual antioxidant. More number of vaso-occlusive episodes is seen in patients with the lowest TAC level.[18] This indicates that the depletion in low molecular weight antioxidants may be conducive for the vaso-occlusive crisis. Various studies have shown the depleted levels of nonenzyme antioxidant molecules such as carotene, Vitamin E, Vitamin C, and trace elements contributing antioxidant activity in SCD patients.[29]

Previous studies have reported significantly decreased levels of tocopherol, retinol, carotenes, ascorbic acid, and zinc.[9,19,30] The deficiency of these antioxidants may account for some of the observed manifestations of sickle cell disease, such as an increased susceptibility to infection and hemolysis.[30] The regular supplementation of these antioxidants may ameliorate some of the sickle cell manifestations such as vaso-occlusive crises, acute chest syndrome, recurrent infection, and growth retardation.[31]

**CONCLUSION**

In the present study, an increased oxidative stress in terms of elevated plasma MDA and decreased serum NO levels was observed. However, a decrease in the antioxidative activity of serum may be due to the overburden by the ROS load. The supplementation of antioxidant vitamins may result in an improved antioxidant activity of serum as well as a decrease in the oxidant levels. The future large randomized controlled studies may be helpful in the development of cost-effective treatment formulation for sickle cell disease in the steady state as well as in the crisis state in resource-poor countries.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Omoti CE. Hematological values in sickle cell anaemia in steady state and during vaso-occlusive crises in Benin City, Nigeria. Ann Afr Med 2005;4:62-7.

2. Olutunji PO. Sickle cell disease in developing countries: Magnitude and challenges. Postgrad Doct (Africa) 2002;25:61-4.

3. WHO. Sickle-Cell Anaemia – Report by the Secretariat. World Health Organization; 2010. p. 11-27.

4. Klings ES, Farber HW. Role of free radicals in the pathogenesis of acute chest syndrome in sickle cell disease. Respir Rev 2001;2:280-5.

5. Airede KI, Ibrahim M. Antioxidants in neonatal systemic disease. Sahel Med J 1999;2:66-72.

6. Fridovich I, Freeman B. Antioxidant defences in the lung. Annu Rev Physiol 1986;48:693-7.

7. Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine. 4th ed. New York: Oxford University Press; 2007. p. 888.

8. Hobbelen RJ, Eaton JW, Balsasingam M, Steinberg MH. Spontaneous oxygen radical generation by sickle erythrocytes. J Clin Invest 1982;70:1253-9.

9. Adelekan DA, Thurnham DI, Adekile AD. Reduced antioxidant capacity in paediatric patients with homozygous sickle cell disease. Eur J Clin Nutr 1989;43:609-14.

10. Giera M, Lingeman H, Niessen WM. Recent advancements in the LC- and GC-based analysis of malondialdehyde (MDA): A Brief overview. Chromatographia 2012;75:433-40.

11. Titas J, Chari S, Gupta M, Parekh N. Pro-oxidant and anti-oxidant status in patients of sickle cell anemia. Indian J Clin Biochem 2004;19:168-72.

12. Das SK, Nair RC. Superoxide dismutase, glutathione peroxidase, catalase and lipid peroxidation of normal and sickled erythrocytes. Br J Haematol 1980;44:87-92.

13. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 1978;90:37-43.

14. Guevara I, Iwanekjo K, Dembisska-Kieć A, Pankiewicz J, Wanat A, Anna P, et al. Determination of nitrite/nitrate in human biological material by the simple griess reaction. Clin Chim Acta 1998;274:177-88.

15. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol 2001;54:356-61.

16. Emokpae AM, Ojiefio UP, Aisha KG. Antioxidant enzymes and acute phase proteins correlate with marker of lipid peroxide in adult Nigerian sickle cell disease patients. J Basic Med Sci 2010;13:177-82.

17. Arinola OG, Olanisya SA, Akibun MO. Evaluation of antioxidant levels and trace elements status in Nigerian sickle cell disease patients with plasmodium parasitaemia. Pak J Nutr 2008;7:766-9.

18. Foluke F, Kayode A, Johan A, Modupe K. Total anti-oxidant status in sickle cell disease patients in steady state. J Natl Med Assoc 2008;100:891-4.

19. Hasanati RM. Zinc and antioxidant vitamin deficiency in patients with severe sickle cell anaemia. Ann Saudi Med 2006;26:17-21.

20. Eberhardt RT, McMahan L, Duffy SJ, Steinberg MH, Perrine SP, Loscalzo J, et al. Sickle cell anemia is associated with reduced nitric oxide bioactivity in peripheral conduit and resistance vessels. Am J Hematol 2003;74:104-11.

21. Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: A novel mechanism of human disease. JAMA 2005;293:1653-62.

22. Reiter CD, Wang X, Tanus-Santos JE, Hogg N, Cannon RO 3rd. Schechter AN, et al. Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. Nat Med 2002;8:1383-9.

23. Morris CR, Kato GI, Poljakovic M, Wang X, Blackwelder WC, Sachdev V, et al. Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. JAMA 2005;294:81-90.

24. Jain SK. The accumulation of malondialdehyde, a product of fatty acid peroxidation, can disturb aminophospholipid organization in the endothelial cell membrane. J Biol Chem 1999;274:3391-4.
Association with phospholipids and potential role in lipid peroxidation. Blood 1988;72:1278-85.
28. Asakura T, Minakata K, Adachi K, Russell MO, Schwartz E. Denatured hemoglobin in sickle erythrocytes. J Clin Invest 1977;59:633-40.
29. Chiu D, Lubin B. Abnormal Vitamin E and glutathione peroxidase levels in sickle cell anemia: Evidence for increased susceptibility to lipid peroxidation in vivo. J Lab Clin Med 1979;94:542-8.
30. Essien EU. Plasma levels of retinol, ascorbic acid and alpha-tocopherol in sickle cell anemia. Cent Afr J Med 1995;41:48-50.
31. Gupta VL, Chaubey BS. Efficacy of zinc therapy in prevention of crisis in sickle cell anemia: A double blind, randomized controlled clinical trial. J Assoc Physicians India 1995;43:467-9.