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# EVALUATION OF GLUTATHIONE PEROXIDASE ACTIVITY, MALONYLALDEHYDE AND SELENIUM LEVELS IN SICKLE CELL ANAEMIA INDIVIDUALS ATTENDING NAUTH, NNEWI IN SOUTH-EASTERN NIGERIA.

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## ABSTRACT

***Background****: Oxidative stress is an imbalance between the production and clearance of toxic free radicals known as reactive oxygen species (ROS). It is associated with hemolysis, a hallmark of SCD. SCD has long been recognized as an inflammatory condition and oxidative stress play important role in pathophysiology of SCA. Imbalance between production and elimination of reactive oxygen species (ROS) damages cell structures, including lipids, membranes, proteins and nucleic acids resulting in cell death or altered cell function.*

***Aim****: The aim of this study was to measure the serum level of Glutathione peroxidase,Malonaldehyde and Selenium in SCA (HbSS) in their steady state, AA (Healthy adult) and AS (sickle cell carriers)*

***Methods:*** *This is a cross- sectional study. A total of one hundred and fifty aged matched participants were recruited for this study.50 SCA subjects with sickle cell anemia (SS). Glutathione peroxidase and Malonyaldehyde were measured using turbidometry and colorimetric methods respectively while Selenium was analysed using Atomic Absorption Spectrophotometry (AAS)*

***Results****: The result of analysis of variance (ANOVA) showed that the mean serum level of MDA concentration (2.97±0.29) was higher in the SS group and was statistically significant ($p< 0.05$). MDA concentration was lower in the AS group. In the post hoc analysis MDA was statistically significant between SS vs AS and AA vs AA. The glutathione peroxidase activity was significantly lower in the SS group (0.85±0.12) compare with the mean values obtained in the AA and AS group. The GPx activity among the three groups was statistically significant while the post hoc analysis among the three groups showed statistical difference and was significant ($p < 0.005$) The Selenium levels in the SS group is lower compared with the values observed between the AA and AS group. The Se levels in the SS group was statistically lower (5.73±1.34) ($p < 0.05$) and showed significant difference. The comparison study between AS vs SS and AA vs AA were statistically significant.*

***Conclusion****: The decrease in selenium level potentiates low and weakened antioxidant activity and capacity which also is a reflection of low level of glutathione peroxidase observed which is supposed to attack free radicals to prevent oxidative stress. The findings from this study are in tandem with other researches suggesting that the pathophysiology of sickle cell disease is associated with oxidative stress*

**KEY WORDS**: *MDA Malonylaldehyde, GPx Glutathione peroxidase, Se Selenium SS Homozygous sickle cell hemoglobin, AA Heterozygous Adult hemoglobin AS Heterozygous sickle disease carrier hemoglobin.*

## INTRODUCTION

Sickle cell anemia (SCA) is a genetic disorder. That affects hemoglobin production and causes red blood cells to change in shape and breakdown faster than normal (1). SCA results from a point mutation in which an adenine nucleobase in the sixth codon of the β globin gene is replaced by a thymine. Translationally replaces glutamic acid with valine thereby producing an abnormal form of hemoglobin S (1-2). Repeated polymerization of hemoglobin S can cause definitive damage to the structure of red blood cells producing mainly intravascular hemolysis. Oxidative stress is an imbalance between the production of free radicals and the antioxidant defense system in favor of free radicals. Free radical has been implicated in the aetiology of over 100 diseases including Sickle Cell Disease (SCD). SCD has long been associated with inflammation and cytotoxicity due to increased radical production. Components of the antioxidant defense systems such as glutathione peroxidase (GPx) and its metallo- enzyme selenium (Se) play crucial roles in mopping up radical species and preventing the deleterious consequences of free radical mediated reactions.

Oxidative stress is enhanced in SCA and would also play a major role in the pathophysiology of the disease by promoting red blood cell (RBC) damage, inflammation, and endothelial-vascular dysfunction (1–4) Elevated advanced oxidation protein products (AOPP) and malondialdehyde (MDA) concentrations have been consistently reported in patients with SCA. (5–12). As a consequence, it would be expected that RBC and serum from individuals with SCA exhibit decreased antioxidant capacities. However, rather inconsistent results were reported by different researches. For instance, Biswal et al. (11) reported lower superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities in SCA individuals compared to controls. Mockesch et al. (7) found higher SOD activity in SCA than in healthy individuals and similar CAT and GPX activities in the two populations. Renoux et al. (2) described higher SOD and lower GPX activities but similar CAT activity in a group of SCA individuals compared to a control group. Finally, Faes et al. [13] found higher GPX activity in young adults with SCA compared to healthy individuals but no difference in SOD and CAT activities. These findings show that a clear picture regarding the activities of antioxidant enzymes is difficult to depict in SCA.

**Aim**; The aim of this study was to evaluate the activity of GPx as well as levels of selenium and radical by-product Malondialdehyde (MDA) in sickle cell anemia (SCA), adult individuals (HbSS) at steady state, Hb AS and Hb AA.
**Methods:** 5ml of venous blood sample was collected from all participants who enrolled for the study and dispensed into plain sterile bottle, allowed to clot, centrifuge at 3,000r.p.m for 10 minutes, serum was separated into another plain sterile container, refrigerated at -20$^0$c until assay.A total of one hundred and fifty participants (150) were recruited for this study and classified into three groups according to their genotypes Hb AA, Hb AS and Hb SS (N =50) respectively. GPx activity was measured using Enzyme linked immunosorbent assay method (ELISA), MDA level was measured using Immunoturbidometric method while Selenium level was measured using atomic absorption spectrophotometry method (AAS). SPSS version 26 was used for the statistical analysis. Ethical approval was obtained from NAUTH ethics committee. Structured questionnaire was filled by each participant and also obtained their informed consent to be voluntarily part of the study. Those who complied with the inclusion criteria were recruited in the study.

## RESULTS

**TABLE 1** LEVELS OF MDA, GPx and Se AMONG THE THREE GENOTYPES (AA, AS, SS)

| GROUP (n=150) | MDA (nmol/l) | GPx (u/ml) | Se(mcg/l) |
|---|---|---|---|
| **SS (50)** | 2.97±0.29 | 0.85±0.12 | 5.73±1.34 |
| **AS (50)** | 2.49±0.22 | 0.92±0.47 | 6.20±1.58 |
| **AA (50)** | 2.87±0.70 | 1.03±0.13 | 7.25±1.78 |
| **f-value** | 15.415 | 36.887 | 12.141 |
| **p-value** | 0.001* | 0.001* | 0.001* |
| **SS vs AS** | 0.001* | 0.002* | 0.003* |
| **SS vs AA** | 0.779 | 0.001* | 0.422 |
| **AS vs AA** | 0.001* | 0.0001* | 0.001* |

The results showed that MDA concentration (2.97±0.29) was higher in the SS group and was statistically significant ($p < 0.05$). MDA concentration was lower in the AS group which is the group of the individuals with the sickle cell trait but does not show clinical manifestation of the disease condition. In the post hoc analysis MDA was statistically significant between SS vs AS and AA vs AA. The glutathione peroxidase activity was significantly lower in the SS group (0.85±0.12) compare with the mean values obtained in the AA and AS group. The GPx activity among the three groups was statistically significant while the post hoc analysis among the three groups showed statistical difference and significant respectively. The Selenium levels in the SS group is lower compared with the values observed between the AA and AS group. The Se levels in the SS group was statistically lower (5.73±1.34) ($p < 0.05$) and showed significant difference. The comparison study between AS vs SS and AA vs AA were statistically significant. However, the Se level didn't show any statistical difference between AA and SS.

Discussion: Oxidative stress is an imbalance between the production and clearance of toxic free radicals known as reactive oxygen species (ROS) is associated with hemolysis, a hallmark of SCD. SCD has long been recognized as an inflammatory condition and oxidative stress play important role in pathophysiology of SCA (5). Imbalance between production and elimination of reactive oxygen species (ROS) can damage cell structures, including lipids, membranes, proteins and nucleic acids resulting in cell death or altered cell function. It is now well established that ROS mediate inflammatory process and may be involved in oxidative reactions such as lipid peroxidation and protein oxidation (6) Although results are sometimes contradictory, Individuals with SCA are shown to have high oxidative stress. Normal RBCs are usually subjected to oxidative stress as a result of continuous ROS production that accompanies Hb autoxidation (23); a condition that increases two times more in SCA (7), leading to a continuous inflammatory response, oxidative stress and associated with endothelial dysfunction, inflammation and multiple organ damage. To counter the destructive effects of these oxidants, there are endogenous antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase (28) which help to detoxify ROS (19). These antioxidants are better known as oxygen radical scavengers. Glutathione peroxidase is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. Glutathione peroxidase (GPx) is a selenium-containing antioxidant enzyme that effectively reduces $H_20_2$ and lipid peroxides to water and lipid alcohols, respectively, and in turn oxidizes glutathione to glutathione disulfide. Reduced glutathione (GSH) is a major tissue antioxidant. In the event of cells exposed to increased oxidative stress, GSH changes to oxidized glutathione (GSSH) and, hence, the ratio of GSH/GSSG decreases. In SCD patients, GSH levels as well as GSH/GSSG ratio are decreased (20). Therefore, measurement of GSH levels may serve as a useful biomarker of ***in vivo*** oxidative stress (5). In addition, GSH and glutamine concentrations are among the indirect markers to assess the oxidative assault on tissues and cells in disorders such as SCD (30)

The Main Reaction That Glutathione Peroxidase Catalyzes Is $2GSH + H_20_2 \rightarrow GS–SG + 2H_20$ where GSH represents reduced monomeric glutathione, and GS– SG represents glutathione disulfide. The mechanism involves oxidation of the selenol of a selenocysteine residue by hydrogen peroxide. Glutathione peroxidase (GPx) catalyzes the reduction of hydrogen peroxide ($H_20_2$) and lipid peroxides by glutathione (GSH). Selenium is present at the active site of GPx as selenoproteins. Glutathione acts as an antioxidant by directly scavenging free radicals through the donation of a hydrogen atom. The synthesis of glutathione is homeostatically controlled, both inside the cell and outside. Low serum selenium levels observed in this study may also suggest that a weakened antioxidant potential may be associated with sickle cell disease patients. This is in line with the study of Nnodim *et al* (2014). Se levels among SCA patients, however, were significantly lower than those of the controls. The findings in this study are also in tandem with a study carried out by Turk *et al* (2019). The levels of Se and glutathione peroxidase were significantly ($p \leq 0.005$) lower than those of controls. These data are consistent with the previous reports that there is increased oxidative stress in SCA. Low blood Se levels and glutathione peroxidase activity observed in this research suggest that a weakened antioxidant potential may be associated with Sickle cell anaemia individuals.

The mean serum selenium level in individuals with sickle cell anaemia in this study was significantly lower compared with the concentration of that obtained from the control which is in tandem with the studies by Hamdy *et al* in Egypt, Olaniyan et al in Oyo state and Idonijie *et al* in Edo state in Nigeria. Reduced selenium levels in SCA have been ascribed to chronic oxidative stress (even in patients in steady state) resulting from increased release of ROS due to repeated cycles of ischaemia and reperfusion, infections and haemolysis. The ROS released overwhelm available antioxidants including selenium. There is also an increased resting metabolic rate and resting energy expenditure in SCA with subsequent increased turnover of macro- and micronutrients and subsequent depletion of micronutrients like selenium which is an important antioxidant. It can then be inferred from this study that there is increased oxidative stress in individuals with SCA as compared to their Hb AA counterparts. The mean selenium levels of individuals with SCA in this study was comparable to the findings in the study by Olaniyan *et al* (5.50±4.0 µg/L) but were lower than the mean values recorded by Nnodim *et al* (60.69±3.12 µg/L) and Idonijie *et al* (60.98±7.29 µg/L). Hamdy *et al* on the other hand had remarkably lower serum selenium levels than what was seen in this study (29.8±20.80 µg/L). These variations in the serum selenium levels reported in all these studies may be a reflection of the fact that serum selenium levels are influenced by intake in the diet which varies based on geographical location.

The mean serum MDA level in the SS group was significantly higher compared to the observed values from the AA and AS group ($p > 0.001$). Also, the mean serum GPX activity in the SS group after was significantly lower compared to the observed values in the control groups ($p < 0.001$). Oxidative damage of red cell membrane is known to be accelerated in SCD. However, the exact mechanism of this acceleration is unclear (Natta *et al*.,). Natta et al reported that a modest decrement of Glutathione peroxidase (GPx) activity could have been partly responsible for accelerated peroxidation of the red cell membrane resulting in hemolytic anemia. GPx contains Se as selenocysteine at its active site. Selenium (Se) deficiency results in decreased enzyme activity. In a study at New Zealand, it was observed that they were haematologically normal inspite of decreased enzyme activity due to low intake of selenium although GPx deficiency was found in healthy controls and Se content in serum was low (26) sickle cell erythrocytes show increased susceptibility to lipid peroxidation.

It was previously reported that GPx protected the membrane against lipid peroxidation in the presence of reduced glutathione by reducing lipid hydroperoxides to lipid alcohols contrary to earlier studies (25) and (21).Conversely, accumulated evidence indicates that GPx is not responsible for the protection against lipid peroxidation in red cells or other tissues (14) this was experimentally confirmed by Grossman and Wendel who showed that phospholipid hydroperoxides are not reduced by GPx (29), Although Ursni et al (28) reported about Se containing GPx which acted upon phospholipidperoxide in membrane structure, it was different from “Classical” selenium glutathione peroxidase.
In a study conducted by Natta et al it was observed that the levels of Se and GPx were significantly lower (less than 0.005) than those of controls in SCD patients in their steady state and this corroborates with the findings in this study. The low Se status in SCA patients may also affect the phenotypic expression of these patients (Natta *et al.,* 1990). GPx is an intracellular antioxidant enzyme that enzymatically reduces $H_20_2$ to water to limit its harmful effect. Se plays an important role during erythropoiesis in which selenoproteins influence the multiple stages of erythroid development, in addition to controlling the oxidative stress that occurs during the process thus promoting adequate cell maturation (26).

Se deficiency may possibly be attributed to a selenium poor diet, since the food sources of selenium are expensive and in Nigeria the population is more vulnerable to food insecurity. In addition, Se deficiency may also be attributed to the increased renal excretion of SCD patients (25). Consequently, tubular reabsorption is known to be abnormal in individuals with SCD leading to loss of nutrients due to the repeated sickling process of red blood cells (2).

Lipid peroxidation is usually assessed in humans by measuring MDA which is one of the most popular and reliable markers that determine oxidative stress in clinical situations (Giera *et al.,* 2012). Increased production of ROS in SCA has been stated to have a role in oxidative stress and induction of lipid peroxidation the byproduct of which is MDA (6). MDA levels were found to be increased in serum of SCA compared with healthy controls. This could be attributed to enhanced ROS formation to SCA (24). MDA in excess quantity can promote erythrophagocytosis. An elevated level of MDA has also been observed in previous studies suggesting the excessive formation of ROS by sickle cells (21,14 and 16) In this study, the serum MDA is significantly elevated in the SS group and thus agrees with other previous findings (21 and 14). Accumulation of MDA disturbs the organization of phospholipids in the human erythrocyte membrane bilayer. The oxidation of phospholipids in the serum, plasma, or blood and internal organelle membranes (eg mitochondria) damage their function. Membrane damage is considered as an important factor contributing towards pathophysiology due to the formation of irreversible sickle cells (5). Red blood cells are particularly susceptible to peroxidative damage because they contain Hb, one of the most powerful catalysts for initiation of the peroxidative reaction (22). It is also stated that excess quantity of MDA can promote erythrophagocytosis to macrophages (8). The HbS red blood cell membranes were exposed to increased amounts of the endogenous oxidant. Hb free iron act as Fenton’s reagents and produces superoxide, peroxide and hydroxyl radicals which may further initiate membrane lipid peroxidation (27). This enhanced oxidative stress may be a contributing factor in the pathogenesis of sickle cell anemias.

Hebbel *et al* have shown that under ambient oxygen tensions sickle cell spontaneously generate oxygen radicals, hydrogen peroxides and hydroxyl radicals approximately two times more when compared with normal red blood cells (25).

## CONCLUSION

The observed reduction in the activity of glutathione peroxidase may be due to increased consumption of enzyme used in combating the free radical load leading to elevation in MDA level which is a product of lipid peroxidation. MDA levels are constantly elevated in SCA, important differences in oxidative stress parameters can be observed from one study to another, depending on environmental, medical, and dietetical differences between cohorts The decrease in selenium level potentiates low and weakened antioxidant activity and capacity which also is a reflection of low level of glutathione peroxidase observed which is suppose to attack free radicals to prevent oxidative stress. The findings from this study are in tandem with other researches suggesting that the pathophysiology of sickle cell disease is associated with oxidative stress. Oxidative stress results from an imbalance between pro-oxidant and antioxidant activities. The rise of some antioxidant enzymes activities in SCA could be interpreted as an expected compensatory response to keep the oxidative stress system in a healthy equilibrium at steadystate.

## CONFLICT OF INTEREST

The authors declare no conflict of interest

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