

Some Antioxidant Enzymes among Children with Sickle Cell Disease Attending Usmanu Danfodiyo University Teaching Hospital Sokoto, North Western Nigeria

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Abstract

Sickle Cell Disease (SCD) is one of the most common genetic diseases in the world. It is associated with oxidative stress which occurs as a result of HbS unstable character causing a rise in the formation of free radicals. The aim of this study was to determine some antioxidant enzymes activities among patients with SCD. We investigated the superoxide dismutase (SOD), and glutathione peroxidases (GPx) levels among 60 children aged 1 - 14 years with SCD. Twenty-two age-matched non-SCD children served as control. The study subjects were divided into two groups; steady state A (n = 30) and vasoocclusive crisis (VOC) B (n = 30). The SOD, and GPx levels were significantly lower among the SCD subjects compared to controls (p = 0.000). There were no statistically significant differences in the SOD and GPX levels between sickle cell disease patient in steady state (A) and those in crisis (B) (p = 0.998)and 0.555) respectively. There was a statistically significant difference between the SOD and GPX levels between sickle cell disease patient in steady state (A) and non-sickle cell controls (p = 0.005 and 0.000) respectively as well as between sickle cell disease patient in VOC (B) and non-sickle cell controls (p = 0.000). There were no statistically significant differences in the SOD and GPX levels of sickle disease subjects based on age, gender, maternal level of educational attainment, occupational group and income (p = 0.629 and0.476; p = 0.382 and 0.417; p = 0.450 and 0.314 and p = 0.397 and 0.762 and p = 0.553 and 0.929) respectively. There were no statistically significant differences in the SOD and GPX levels of sickle disease subjects of Hausa/Fulani extraction versus Yoruba (p = 0.714 and 0.856), between Hausa/Fulani extraction versus Igbo (0.917 and 0.486) and between Yoruba extraction versus Igbo (p = 0.740 and 0.965) respectively. This study confirms that SCD children have lower values of antioxidant enzymes compared to controls. SOD and GPX levels in sickle cell disease patient in steady state and vaso-occlusive crisis are significantly lower compared that of non-sickle cell controls. Patients with SCD may benefit from substances with antioxidant properties which can potentially reduce the complications associated with the disease.

Keywords

Antioxidant Enzymes, Children, Sickle Cell Disease, Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria

1. Introduction

SCD as one of the most common genetic causes of illness and death in the world [1]. In HbS, there is substitution of one amino acid (valine) for another amino acid (glutamic acid) at position six of the β -globin polypeptide chain at codon 6 within the β -globin gene on chromosome 11. It affects approximately 5% - 7% of the world's population with the highest prevalence in the Middle East, Mediterranean regions, Southeast Asia, and Sub-Saharan Africa [2]. It affects about 10% of the population in Ghana while in Nigeria approximately 25% of the population of 198 million has sickle cell trait and 3% has SCD [3]. It is currently estimated that 3.6% of population in Yoruba land of Western Nigeria has SCD [4].

SCD arises from the inheritance of two abnormal haemoglobin genes, one from each parent. It occurs due to mutation in the haemoglobin gene [5] [6]. The mutation in the haemoglobin gene causes the production of RBCs that are sickled or crescent shaped, sticky, and rigid, instead of the normal biconcave disc seen in RBCs containing normal adult haemoglobin A. This makes the cells clump together and block the blood vessels, inadvertently blocking the flow of blood and affecting oxygen supply throughout the body [3] [7]. These RBCs have a shorter life-span when compared to normal RBCs, causing them to collapse and break down easily [8]. The sickled RBC reduces its ability to polymerize and disturbance in the red cell membrane makes the cell less responsive to oxidant stress, and altered membrane lipids resulting in increased rigidity which leads to haemolysis [9].

Oxidative stress, which is often experienced by SCD patients occur as a result of continuous production of reactive oxygen species (ROS), may lead to endothelial dysfunction and acute inflammation. A high production rate of reactive oxygen species (ROS) in SCD is caused by factors such as increased intrvascular hemolysis, ischemia-reperfusion injury, and chronic inflammation. It is the oxidative damage in sickled RBCs that leads to haemolysis which causes an increase in the formation of free radicals in association with reduced antioxidant defense mechanisms, leading to increased generation of oxidation products [10]. Free radicals are potentially harmful, and several host defense mechanisms are in place to neutralize their effects, which are either produced naturally in the body (endogenous antioxidants), or externally via food supplementation (exogenous antioxidants). Endogenous antioxidants include antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) and glucose 6-phosphate dehydrogenase (G6PD) which is considered as the primary defensive system of the cell. These antioxidants act to counteract additional formation of free radicals, to protect the cells against their destructive and noxious effects and to contribute to disease prevention [10].

Oxidative stress is defined as an imbalance between free radicals and antioxidants balance leading to potential cellular damage. Abnormal oxidant/antioxidant balance is implicated in the pathophysiology of several dysfunctions observed in SCD patients, especially during VOC, resulting in various haematological and biochemical changes. The iron released during haemolysis of the RBC is one of the major contributors to the increased ROS production in SCD. SOD is one of the most important enzymatic antioxidants. They catalyse the conversion of superoxide to hydrogen peroxide and molecular oxygen [11]. Glutathione peroxidases (GPx) are essential components of cellular detoxification systems that defend cells against reactive oxygen species (ROS) [12]. GPx provides 2^{nd} line of defense against ROS [13]. Glutathione (GSH) is the most abundant thiol that can directly scavenge free radicals or act as substrate for (GPx) during detoxification of H₂O₂ and electrophilic compounds.

Approximately 150,000 Nigerian children are born each year with SCD, making the country one with the largest burden of SCD in the world [14]. This high burden of SCD is due to the population in Nigeria of over 198 million by the National Population Commission 2018. It is currently estimated that about 25% of adult Nigerians have sickle cell trait and 3% have SCD [3]. However, a study done in Sokoto [15] shows that 12.5% of patients attending paediatrics clinic in Usmanu Danfodio University Teaching Hospital (UDUTH) Sokoto, North Western Nigeria have SCD. SCD accounts for up to 20% of neonatal mortality [1] [16]. In 2006, the World Health Organization declared SCD to be a problem of major public health significance and a burden that must be addressed if recent improvements in overall child survival are to be consolidated [17].

Although several studies have been carried out on the aforementioned parameters across the globe, there is paucity of data on antioxidant enzymes level among SCD patient in Sokoto, North Western Nigeria. It is hoped that this study will generate evidence-based data that will assist in the management of SCD. The aim of this study is to determine the levels of some antioxidant enzymes (SOD, GPx) among children with SCD in Sokoto, North Western Nigeria.

2. Materials and Methods

2.1. Study Area

The study was carried out in the Paediatric Outpatient Department of Usmanu

Danfodiyo University Teaching Hospital (UDUTH) Sokoto and Specialist Hospital Sokoto. The hospitals are located within the Sokoto metropolis, in Sokoto State. Sokoto State occupies 25,973 square kilometers and is situated along latitude 13°3'39"N and longitude 5°14'2"E. The state is located in the extreme North Western corner of Nigeria near the confluence of the Sokoto River and the Rima River. It shares borders with Niger Republic to the North, Zamfara State to the East, Kebbi State to the South-East and Benin Republic to the West. The major indigenous tribes in the state are the Hausa and Fulani and other groups such as Gobirawa, Zabarmawa, Kabawa, Adarawa, Arawa, Nupes, Yorubas, Igbos and others. The Majority of the Hausas' are farmers while Fulanis are nomadic and are engaged in animal rearing. Sokoto is a very hot area located in the dry Sahel, surrounded by sandy savannah and isolated hills, with an annual average temperature of 28.3°C (82.9°F). However, during the dry season daytime temperature is about 40° C (104.0°F). With annual growth rate of 3%, Sokoto state has a population of 4.2 million as of 2006 [18]. The calculated projected population for Sokoto State is now standing at around 5.3 million. The state is a major commerce center in leather, crafts and agricultural products.

2.2. Sample Size Calculation

The sample size was determined using the formula $(z^2 pq/d^2)$ [19].

- *n* = minimum sample size.
- *z* = standard normal deviation and probability.
- *p* = prevalence or proportion of value to be estimated from previous studies.
- q = Proportion of failure (=1 P).
- d = precision, tolerance limit, the minimum is 0.05.

Therefore, $n = z^2 pq/d^2$.

Where, Z = 95% (1.96).

P = 3% (0.03) [3]. q = 1 - 0.03 (=0.97). d = 5% (0.05). Therefore, $n = (1.96)^2 (0.03) (0.97) / (0.05)^2$. n = 45.

2.3. Study Population

The study population included 60 consecutively-recruited children with SCD (30 on crisis and 30 on steady state) aged 1 - 14 years (subjects) while 22 age and gender-matched children with haemoglobin AA were monitored as controls. Both subjects and controls were recruited consecutively from UDUTH and Specialist Hospital Sokoto, North-Western Nigeria.

2.4. Inclusion Criteria

Subjects with confirmed haemoglobin-SS, age (1 - 14 years), and willingness of parents/guardians to offer verbal informed consent for their ward to participate

in the study.

2.5. Exclusion Criteria

The following who did not meet the inclusion criteria were excluded from the study; SCD children > 14 years and < 1 year old, SCD children who had a recent transfusion in the last 4 months, SCD children whose parents or guardian refused to provide verbal informed consent for their ward to participate in the study.

2.6. Study Design

This research was a case-control study involving age and gender-matched children who were homozygous for HbSS (subjects) and HbAA (controls). Sociodemographic data of the patients were obtained by using an intervieweradministered questionnaire which included the age, gender and other socio-demographic factors. Quantitative data was obtained by estimating the SOD and GPX levels. The study was carried out between January and September, 2018.

2.7. Ethical Considerations

Ethical approval was obtained from the ethical committee of Usmanu Danfodiyo University Teaching Hospital (UDUTH) and Specialist Hospital, Sokoto. Verbal informed consent was obtained from the parents or guardians of the subjects prior to the commencement of the study.

2.8. Sampling Techniques

Sample Collection

Whole blood was collected via venipuncture using BD vacutainer system into plain tubes under strict aseptic techniques. The samples were allowed to clot and the clotted blood sample was centrifuged at 3000 rpm for ten minutes on a bench-top centrifuge. The sera obtained was transferred into sterile cryovials and stored at -20° C immediately until ready to be analyzed. The laboratory analyses were conducted at the Haematology Laboratory UDUTH Sokoto, Nigeria. The serum was used for the assay of SOD and GPX.

Methods of Analysis

Enzyme-Linked Immunosorbent Assay (ELISA) was used for the analysis of SOD and GPX.

Determination of SOD and GPx

SOD was estimated using ELISA technique (Melsin Medical Co., Limited, China). This test uses enzyme-linked immunosorbent assay-double antibody sandwich principle to assay the SOD level in the sample. The Microelisa strip plate has been coated by Purified SOD antibody to make solid-phase antibody. When the sample containing SOD is added to wells, it combines with SOD antibody labelled by Horse Radish Peroxidase (HRP), and become antibody-antigen and enzyme-antibody complex. The non-combined enzyme is washed completely and Chromogen Solution A and Chromogen Solution B are added, which makes the color of the liquid to change to blue. The acidic solution is added and the colour finally becomes yellow. The colour change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of SOD in the samples is then determined by comparing the O.D. of the samples to the standard curve. GPx was estimated using ELISA technique (Melsin Medical Co., Limited, China). This test uses enzyme-linked immunosorbent assay-double antibody sandwich principle to assay GPx levels in the sample. The Microelisa strip plate has been coated by Purified GPx antibody to make solid-phase antibody. When the sample containing GPx is added to wells, it combines with GPx antibody labelled by Horse Radish Peroxidase (HRP) and become antibody-antigen and enzyme-antibody complex. The non-combined enzyme was washed completely and Chromogen solution A and Chromogen solution B were added, which made the colour of the liquid to change to blue. The acidic solution was added and the colour eventually becomes yellow. The colour change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of GPx in the samples was then determined by comparing the O.D. of the samples to the standard curve.

2.9. Statistical Analysis

Statistical analysis was performed using statistical package for social sciences (SPSS) version 20. Frequencies and percentages were calculated. Student t-test (independent t-test and paired sample t-test) and ANOVA were used for comparison of data. The results were presented as mean \pm standard error of mean. A p-value of ≤ 0.05 was considered as significant in all statistical comparisons.

3. Results

Table 1 shows the socio-demographic characteristics of SCA children (steady and crises) and control group. Majority of SCD children were aged 5 years and above, with 80% in steady, 50% in crisis and 68.2% in control group. There was equal distribution of males and females (50%) in steady state group but a slight increase in the number of females compared to males among SCD subjects in crisis and among the control group (56.7% and 68.2% respectively). Ethnic distribution indicated that Hausa/Fulani accounted for 90% SCD children and controls. Distribution based on the maternal level of education indicated that most of the maternal parents were either secondary school leavers (40.0% for steady, 33.3% for crisis) or did not have any formal education (23.3% for steady and 33.3% for crisis). The maternal parents are mostly traders with an income of <18,000 naira monthly (53.3% for steady and 83.3% for crises). **Table 2** shows the levels of the antioxidant enzymes of Sickle cell disease children and control individuals. The result showed a statistically significant difference in the SOD and GPX levels between the SCD subjects and controls (p = 0.000). The antioxi-

dant enzymes were compared among SCD subjects (steady and crisis) and control individuals. There were no statistically significant differences in the SOD and GPX levels between the SCD subjects in the steady state (A) and those in crisis (B) (p = 0.998 and 0.555) respectively. There was a statistically significant difference between the SOD and GPX levels between of sickle cell disease patient in steady state (A) and non-sickle cell controls (p = 0.005 and 0.000) respectively. Similarly, there was a statistically significant difference between the SOD and GPX levels between sickle cell disease patient in VOC (B) and non-sickle cell controls (p = 0.000). Table 3 shows the comparison of the antioxidant enzymes among SCD subjects (steady and crisis) and control individuals. Table 4 shows the effect of age on the SOD and GPX levels of sickle disease subjects. There were no statistically significant differences in the SOD and GPX levels of sickle disease subjects based on age (p = 0.629 and 0.476) respectively. Table 5 shows the effect of gender on the SOD and GPX levels of sickle disease subjects. There were no statistically significant differences in the SOD and GPX levels of sickle disease subjects based on gender (p = 0.382 and 0.417) respectively. Table 5 shows the comparison of antioxidant enzymes based on gender of sickle cell disease children. Table 6 shows the effect of ethnicity on the SOD and GPX levels of sickle disease subjects based on ethnicity. There were no statistically significant differences in the SOD and GPX levels of sickle disease subjects of Hausa/Fulani extraction versus Yoruba (p = 0.714 and 0.856), between Hausa/Fulani extraction versus Igbo (0.917 and 0.486) respectively and between Yoruba extraction versus Igbo (p = 0.740 and 0.965) respectively. Table 7 shows the effect of maternal level of educational attainment on the SOD and GPX levels of sickle disease subjects. There were no statistically significant differences in the SOD and GPX levels of sickle disease subjects based on maternal level of educational attainment (p = 0.450 and 0.314) respectively. Table 8 shows the antioxidant enzymes of the SCD subjects based on maternal occupation. There were no statistically significant differences in the SOD and GPX levels of sickle disease subjects based on the occupation groups of the mothers (p = 0.397 and 0.762) respectively. Table 9 shows the antioxidant enzymes levels among the SCD subjects based on maternal income. There were no statistically significant differences in the SOD and GPX levels of sickle disease subjects based on the income of the mothers (p = 0.553 and 0.929) respectively.

4. Discussion

SCA is one of the most common genetic diseases in the world. Approximately 150,000 Nigerian children are born each year with SCD, making it the country with the largest burden of SCD in the world [14] [20]. It accounts for up to 20% of neonatal mortality [1] [16]. In 2006, the World Health Organization declared SCD to be a problem of major public health significance and a burden that must be addressed if recent improvements in overall child survival are to be consolidated [17]. However, there is paucity of data on antioxidant enzymes among

SCD children in Sokoto. The present study investigated some antioxidant enzymes (SOD, GPx) among homozygous sickle cell subjects compared with normal haemoglobin AA controls.

| Group | Steady | % | Crisis | % | Control | % |
|---------------------------|--------|------|--------|------|---------|------|
| N | 30 | | 30 | | 22 | |
| Age (Years) | | | | | | |
| <5 Yrs | 6 | 20 | 15 | 50 | 7 | 31.8 |
| 5 Yrs Above | 24 | 80 | 15 | 50 | 15 | 68.2 |
| Gender | | | | | | |
| Male | 15 | 50 | 13 | 43.3 | 7 | 31.8 |
| Female | 15 | 50 | 17 | 56.7 | 15 | 68.2 |
| Ethnicity | | | | | | |
| Hausa/Fulani | 27 | 90.0 | 27 | 90.0 | 20 | 90.9 |
| Yoruba | 0 | 0.0 | 2 | 6.7 | 2 | 9.1 |
| Igbo | 3 | 10.0 | 1 | 3.3 | 0 | 0.0 |
| Level of Education Mother | | | | | | |
| Primary | 3 | 10.0 | 6 | 20.0 | 0 | 0.0 |
| Secondary | 12 | 40.0 | 10 | 33.3 | 6 | 31.8 |
| Tertiary | 8 | 26.7 | 4 | 13.3 | 15 | 63.6 |
| Non formal | 7 | 23.3 | 10 | 33.3 | 1 | 4.5 |
| Mother's Income | | | | | | |
| <18,000 | 16 | 53.3 | 25 | 83.3 | 6 | 27.3 |
| 25,000 - 40,000 | 2 | 6.7 | 1 | 3.3 | 1 | 4.5 |
| 50,000 - 100,000 | 2 | 3.3 | 0 | 0.0 | 4 | 27.3 |
| >100,000 | 2 | 6.7 | | | 0 | 0.0 |

 Table 1. Socio-demographic characteristics of Sickle Cell Disease subjects and controls.

Key: N = number of subjects, % = Percentage. Data were analyzed using frequency distribution.

 Table 2. Comparison of Antioxidant enzymes of SCD children and apparently healthy children.

| Group | N | SOD (ng/ml) | GPX (pg/ml) |
|---------|----|----------------|------------------|
| SCD | 60 | 33.09 ± 3.05 | 15.54 ± 0.67 |
| Control | 22 | 51.95 ± 3.11 | 26.44 ± 0.88 |
| p-value | | 0.000 | 0.000 |

Key: SOD = Superoxide Dismutase, GPx = Glutathione Peroxidase, N = number of subjects, SCD = Sickle Cell Disease, S = Significant, Correlation is significant at level of ≤ 0.05 . Data were analyzed using student t-test and the results are presented as mean \pm SEM.

| Group | N | SOD (ng/ml) | GPX (pg/ml) |
|----------|----|------------------|------------------|
| А | 30 | 33.08 ± 5.62 | 15.94 ± 1.13 |
| В | 30 | 33.09 ± 2.51 | 15.14 ± 0.74 |
| С | 22 | 51.95 ± 3.11 | 26.44 ± 0.88 |
| Post HOC | | | |
| AVB | | 0.998 | 0.555 |
| AVC | | 0.005 | 0.000 |
| BVC | | 0.000 | 0.000 |

Table 3. Comparison of the Antioxidant enzymes among SCD Children (steady and crisis) and control individuals.

Key: ANOVA = Analysis of Variance, SOD = Superoxide Dismutase, GPx = Glutathione Peroxidase, N = no. of subjects, ng/ml = nanogram per milliliter, pg/ml = Picogram per milliliter, nmol/l = nanomole per litre, A = Steady state, B = Crisis, C = Control, V = Versus, Data were analyzed using one-way ANOVA with turkey post-hoc test. The results are presented as mean ± SEM.

 Table 4. The comparison of antioxidant enzymes based on the age of sickle cell disease subjects.

| Age Group | | | | | | |
|-------------|----------------|----------------|---------|--|--|--|
| Parameter | <5 Years | 5 Years Above | p-value | | | |
| N | 21 | 39 | | | | |
| SOD (ng/ml) | 34.89 ± 3.77 | 32.12 ± 4.26 | 0.629 | | | |
| GPX (pg/ml) | 16.19 ± 1.10 | 15.19 ± 0.85 | 0.476 | | | |

Key: N = no. of subjects, SOD = Superoxide Dismutase, GPx = Glutathione Peroxidase ng/ml = nanogram per milliliter, pg/ml = Picogram per milliliter. Data were analyzed using student t-test and the results are presented as mean \pm SEM.

 Table 5. Comparison of antioxidant enzymes between male and female sickle cell disease subjects.

| Male $N = 28$ | Female $N = 32$ | p-value |
|----------------|---|---|
| | | |
| 30.32 ± 2.80 | 35.52 ± 5.18 | 0.382 |
| 14.97 ± 0.70 | 16.04 ± 1.10 | 0.417 |
| | Male N = 28 30.32 ± 2.80 14.97 ± 0.70 | Male N = 28 Female N = 32 30.32 ± 2.80 35.52 ± 5.18 14.97 ± 0.70 16.04 ± 1.10 |

Key: N = no. of subjects, SOD = Superoxide Dismutase, GPx = Glutathione Peroxidase, ng/ml = nanogram per milliliter, pg/ml = Picogram per milliliter. Data were analyzed using student t-test and the results are presented as mean \pm SEM.

Table 6. The comparison of antioxidant enzymes of sickle cell disease children based on ethnicity.

| Ethnic Group | | | | | | | |
|--------------|---------------------|----------------|---------------|----------|-------|-------|--|
| Group | Hausa/Fulani N = 54 | Yoruba N = 2 | Igbo N = 4 | Post Hoc | | | |
| Parameters | | | | H/F V Y | ΗVΙ | Y V I | |
| SOD (ng/ml) | 32.60 ± 3.25 | 47.21 ± 30.29 | 33.94 ± 11.53 | 0.714 | 0.917 | 0.740 | |
| GPX (pg/ml) | 15.37 ± 0.71 | 17.00 ± 7.13 | 17.39 ± 2.52 | 0.856 | 0.486 | 0.965 | |

Key: N = no. of subjects, SEM = Standard Error of Mean, ANOVA = Analysis of Variance, SOD = Superoxide Dismutase, GPx = Glutathione Peroxidase, ng/ml = nanogram per milliliter, pg/ml = Picogram per milliliter, H = Hausa/Fulani, Y = Yoruba, I = Igbo, V = VS. Data were analyzed using one-way ANOVA with turkey post-hoc test. Results are presented as mean \pm SEM.

Table 7. Antioxidant enzymes among the SCD subjects based on maternal level of education.

| Educational level | | | | | | | |
|--|----------------|----------------|------------------|------------------|-------|--|--|
| Primary Secondary Tertiary Non-formal p-va | | | | | | | |
| Parameters | | | | | | | |
| SOD (ng/ml) | 27.68 ± 3.24 | 41.96 ± 5.72 | 43.75 ± 3.85 | 29.16 ± 2.89 | 0.450 | | |
| GPX (pg/ml) | 13.88 ± 0.85 | 19.29 ± 1.37 | 21.56 ± 1.32 | 14.96 ± 1.24 | 0.314 | | |

Key: ANOVA = Analysis of Variance, SOD = Superoxide Dismutase, GPx = Glutathione Peroxidase, ng/ml = nanogram per milliliter, pg/ml = Picogram per milliliter. Data were analyzed using one-way ANOVA and results are the results are presented as mean ± SEM.

| Table 8. Antioxidant enzymes among S | SCD children ba | ased on maternal | occupation. |
|--------------------------------------|-----------------|------------------|-------------|
|--------------------------------------|-----------------|------------------|-------------|

| | | Occupation | | |
|-------------|------------------|------------------|------------------|-------|
| Occupation | Business | Civil service | House wives | |
| Parameters | | | | |
| SOD (ng/ml) | 35.77 ± 3.92 | 49.34 ± 5.41 | 34.72 ± 2.77 | 0.397 |
| GPX (pg/ml) | 16.26 ± 0.91 | 23.94 ± 1.63 | 19.00 ± 1.44 | 0.762 |

Key: ANOVA = Analysis of Variance SOD = Superoxide Dismutase, GPx = Glutathione Peroxidase, ng/ml = nanogram per milliliter, pg/ml = Picogram per milliliter. Data were analyzed using one-way ANOVA. Results are presented as mean ± SEM.

Table 9. Antioxidant enzymes of SCD children based on maternal income.

| | Maternal | Income | | | | |
|-------------|-----------------|----------------|----------------|--------------|--------------|---------|
| <12 | 8,000 25 - 40,0 | 00 50 - 100,00 | 0 | >100,000 | None | p-value |
| Parameters | | | | | | |
| SOD (ng/ml) | 36.82 ± 3.66 | 54.19 ± 19.08 | 53.07 ± 6.76 | 38.11 ± 8.50 | 32.26 ± 2.82 | 0.553 |
| GPX (pg/ml) | 16.74 ± 0.89 | 19.67 ± 3.72 | 24.74 ± 2.05 | 23.39 ± 3.98 | 18.61 ± 1.63 | 0.929 |

Key: ANOVA = Analysis of Variance, Data is presented as mean \pm SEM, SOD = Superoxide Dismutase, GPx = Glutathione Peroxidase, ng/ml = nanogram per milliliter, pg/ml = Picogram per milliliter. Data were analyzed using one-way ANOVA. Results are presented as mean \pm SEM.

Our study showed that majority of the participants were aged 5 years and above, with 80% in steady, 50% in crisis. The age group of our cohort of SCD subjects in this study is similar to subjects studied in previous reports [21] [22] [23] [24]. Life expectancy of SCA patients is reduced considerably among younger children and older people. This may be because SCD-related complications reduce as the child grows into his adult age and are higher in smaller children and older patients. This indicates that SCD-related mortality is likely to be higher among younger children and older people despite advances that emerged for the prevention and treatment of complications of the disease [25].

With respect to gender, we observed an equal distribution of males and females (50%) among subjects in steady state group but a slight increase of females than males in crisis group (56.7%). This finding is in accordance with some previous reports [22] [24] [26]. This observation may be due to the fact that females generally report or complain more than males when they are suffering from different types of illnesses [27].

We observed that a significant number of the SCA subjects were of the Hausa/Fulani ethnic group (90%). This could be due to the fact that the ethnic background of the study population is predominantly Hausa/Fulani.

Classification based on the level of education attainment of the maternal parents indicated that majority were either secondary school leavers (40.0% for steady, 33.3% for crisis) or had no formal education (23.3% for steady and 33.3% for crisis). The maternal parents were mostly on a low income of <18,000 naira monthly (53.3% for steady and 83.3% for crises). Our finding is consistent with previous reports [28] [29] [30] [31]. The reason why lower levels of education (income) are associated with higher numbers of SCD children in crisis may be because education provides the knowledge and information needed in the management, treatment and coping with the disease. Better educated parents are more likely to be better informed about SCD and its management and outcomes [32] [33]. Higher levels of education and income would mitigate the deleterious effects of the psychosocial consequences (anxiety and depression) associated with SCD. Parents of SCD children with higher education or income are more likely to make better informed decision about nutritional requirements and use of insecticide-treated mosquito nets to protect their children from malaria. They are also more able to afford the medication and the various treatment options required for the effective management of their SCD children [34] [35].

In this study, there was a significant decrease in the activity of antioxidant enzymes (SOD and GPx) among the SCD subjects when compared with haemoglobin AA control. However, no statistically significant difference was observed when SOD and GPx of SCA subjects in the steady state were compared with those in crises group (p = 0.998 and 0.555) respectively. This observation is consistent with previous reports [36]-[40] and may be an indication that SCD subjects produced greater quantities of reactive oxygen species than HbAA controls. Our finding is also consistent with previous reports [10] [36] [41] [42] [43] which indicated that the levels of antioxidant enzymes tend to decrease in sickle cell patients. HbS containing red blood cells auto-oxidize faster thereby generating more superoxide, hydrogen peroxide, hydroxyl radicals and lipid peroxides than HbAA containing red blood cells [10] [44] [45]. Oxidative damage is due to imbalance between the production of reactive oxygen species and the countering effect of the various antioxidants present in the body. In SCD, the production of reactive oxygen species can be grossly amplified in response to variety of pathophysiological conditions such as inflammation, immunologic disorders, hypoxia, metabolism of drugs or alcohol and deficiency in antioxidant enzymes [46]. An increase in these enzymes' activity potentially constitutes a defense mechanism in response to increased oxidative stress or might be a consequence of increased reticulocyte content in blood samples from patients with sickle cell disease.

However, a decrease in enzyme levels tends to be related to disease severity in SCD patients [47]. The decrease seen in our study may be due to the consumption of these substances by pro-oxidants in SCD. This, therefore, places SCD children at increased risk of oxidative stress and injury. The oxidative stress may contribute to the sickling process with formation of dense cells, the development of vaso-occlusion and shortened red blood cells survival [48]. Reactive oxygen species can also cause damage to biological macromolecules and membrane lipids that readily react and undergo peroxidation. The peroxidative process yields lipid peroxides, lipids alcohol and aldehydic products and MDA [49] [50].

Oxidative stress has been shown to play a significant role in the pathophysiology of SCD and its associated complications [51] [52] [53]. Several mechanisms have been postulated to contribute to the increased oxidative burden in SCD patients. Some of the mechanisms include; excessive level of cell-free haemoglobin resulting from haemolysis [54], chronic pro-inflammatory state [52], recurrent ischemic-reperfusion injury [55], higher auto-oxidation of HbS [56] and excess iron [57].

Findings from this study may be a justification to routinely prescribe vitamins for patients with SCD. Previous report indicates that vitamins C and E combined supplement reverses the reduced levels of free radical scavenging enzymes (SOD and GPx) activity in SCA subjects [37].

5. Conclusion

This study confirms that SCD subjects have lower values of serum activity of antioxidant enzymes (SOD, GPx) compared to controls. This study seems a justification to routinely monitor the activity of antioxidant enzymes level of subjects with SCD. Patients with SCD may benefit from substances with antioxidant properties which can potentially reduce the complications of this disease.

Recommendations

We recommend that substances or supplements with antioxidant properties be routine prescribed for patients with SCD to reduce the complications of this disease. Socio-demographic characteristics should be considered seriously in the management of SCD patients. There is a need for the Nigerian government to invest more on the creation of awareness about SCD and its implication in this locality and provide financial assistance and better health care for SCD patients. Parents and guardians of SCD children should be educated on the nutritional requirement for SCD patients to reduce the incidence of VOC and maintain patients in the steady.

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Limitations

Due to cost implication we could not test our subjects and control participants for the broad spectrum of antioxidant enzymes. There is need for an enlarged study to investigate the full spectrum of antioxidant enzymes among Children with Sickle Cell Disease across Nigeria.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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