

Comparism of Antioxidants Status among Children, Teenagers and Adults with Sickle Cell Anaemia.

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Abstract

Background: One of the diseases caused by a mutation in a gene is sickle cell anaemia. Sickle cell anaemia is a serious disorder in which the body makes sickle-shaped red blood cells. The sickle cells block blood flow in the blood vessels, this causes pain as well as organ damage, and it can also raise the risk of infection.

Materials and methods: This study was carried out to determine, as well as compare, the activities of enzymatic antioxidants- Glutathione peroxidase (GPx), Catalase (Cat) and Superoxide dismutase (SOD), as well as some non- enzymatic antioxidants (Vitamins C and E), in the plasma of sickle cell , non-sickle cell and sickle cell trait children, teenagers and adults. Sixty adults were grouped into 3 groups of 20 subjects each; the groups were having the status of AA, AS, and SS respectively. The same grouping arrangement was made for 60 teenagers and 60 children respectively. The subjects for the study were gotten from Federal medical center, Ido Ekiti and some selected hospitals in Ekiti state. All the antioxidants were determined using standard laboratory methods respectively.

Results: The results obtained showed significant reductions ($P < 0.05$) in the concentrations of all the antioxidants in the sickle cell subjects, at all ages - children, teenagers and adults. **Conclusion:** This study suggests that both enzymatic and non-enzymatic antioxidants are implicated in sickle cell anaemia irrespective of the stage and age of the carrier.

Keyword: Sickle cell anaemia, enzymatic antioxidants, Vitamin C, Vitamin E

I. INTRODUCTION

Sickle cell anemia (SCA) is a multi-system disease, associated with episodes of acute illness and progressive organ damage.(Weatherall *et al*,2005). Sickle cell anemia is an inherited blood disorder affecting approximately 5% of the world's population. SCA arises from a p- mutation in the genetic code such that glutamic acid is replaced by valine in the globin chain of hemoglobin. This transforms normal adult hemoglobin (HbA) into sickle hemoglobin (HbS). When deoxygenated, HbS polymerizes, and when a critical amount of HbS polymer accumulates within a sickle erythrocyte, cellular injury occurs. A sufficient number of damaged erythrocytes cause the phenotype of sickle cell disease (SCD), characterized by hemolytic anemia and vasoocclusion (Steinberg *et al*, 2008).

Congenital hemoglobin mutations may alter the delicate balance of free-radical generation and antioxidant defense systems in the red cell resulting in oxidative stress, which constitute a critical factor in endothelial dysfunction, inflammation, and multiple organ damage in SCD. Sickle cell anemia patients are subjected to chronic oxidative stress which is able to cause oxidative damage in biological macromolecules such as DNA, protein and lipids (Oyeyemi *et al*, 2016) ,this is as a result of overproduction of reactive oxygen species (ROS) (Tonetti *et al*, 2005). Futhermore, decreased RBC's elasticity is central to the pathophysiology of sickle cell disease. There is low oxygen tension which promotes RBC sickling and repeated episodes of sickling damages the cell membrane and makes it rigid. Repeated sickling and unsickling generates free radicals, which can irreversibly damage cell wall increasing its rigidity and thus shortening its life span.(Berliner *et al*, 2004) .Thus, oxidative stress may play a role in the pathophysiology of the clinical manifestations of the disease . Antioxidant defense mechanisms against the harmful effects of ROS involve cellular and extracellular enzymes such as catalase, superoxide dismutase (SOD), glutathione reductase and peroxidase and free radical quenchers such as glutathione, vitamin C, vitamin E, carotenoids, albumin, and products of metabolism such as uric acid and bilirubin. (Wood *et al*, 2007).

Catalase is a heme-containing enzyme that catalyzes the degradation of hydrogen peroxide to water and molecular oxygen, it controls the hydrogen peroxide levels so that this does not reach toxic and harmful levels that could result in oxidative damage to the cells.(Aslan *et al*, 2000). Vitamin E is a lipid soluble chain breaking antioxidant, which has protective role in almost all cells of the body. It scavenges free radical by its ability to transfer phenolic hydrogen to a peroxy free radical of peroxidized polyunsaturated fatty acids. (Aslan *et al*, 2000). Vitamin C, a free radical scavenger, directly accepts electron from superoxide hydroxyl anion as well as from various lipid hydroxyl peroxides .

The sickle-cell trait is now known to be widespread, reaching its highest prevalence in parts of Africa, in countries such as Cameroon, Republic of Congo, Gabon, Ghana and Nigeria, the prevalence is between 20% and 30% (WHO, 2015). Very few studies have been carried out on the status of anti-oxidants in different age range of SCA patients in this region. The present study aimed to compare antioxidants status among children, teenagers and adults with sickle cell anaemia in Nigeria.

II. MATERIALS AND METHOD

Selection Of Subject: This was a cross sectional case control study involving 60 adults grouped into 3 groups of 20 subjects each; the groups were having the status of AA, AS, and SS respectively. The same grouping arrangement was made for 60 teenagers and 60 children respectively. The total participants were 180, they were recruited from Ekiti State University Teaching, Ado Ekiti State and Federal Medical Centre, Ido Ekiti. Anthropometric data were collected and informed consent form duly signed.

Sample Collection

The blood samples used for the test were collected from sickle celled patients and non-sickle celled patients at Ekiti State University Teaching Hospital, Ado-Ekiti, and Federal Medical center, Ido-Ekiti, Ekiti State, Nigeria. Intravenous blood (5 ml) was collected from the patients using a sterile syringe. Samples for Total Antioxidants Status (TAS) were drawn in to serum bottles. They were allowed to clot; serum was separated and stored at -20°C. TAS was assessed within two weeks of collection.

Antioxidant Assay

Estimation of SOD Activity

Superoxide dismutase (SOD) activity was determined by the method of Beauhamp and Fevovich (1976).

Estimation of Catalase

Catalase activity was determined by the method described by Ravhkrishnan and Sarma (1963).

Estimation of Glutathione Peroxidase

Glutathione Peroxidase activity was determined by the method described by Paglia and Valantine (1967)

Estimation of Vitamin E

Vitamin E levels were determined by the method of Baker and Frank, (1968).

Estimation of Vitamin C

Vitamin C levels were determined by the method of Roe, (1961).

Statistical Analysis

The results obtained was grouped and expressed as mean \pm Standard Error of Mean (SEM). The data collected was analyzed using one-way Analysis of variance (ANOVA) and Duncan multiple range test to compare the data obtained from the experiment to those of the control (Zar, 1986).

III. RESULTS

Table 1 shows the activities of superoxide dismutase, catalase glutathione peroxidase, vitamin C, vitamin E in sickle cell children and non-sickle cell children. There was significant decrease in SOD activity in sickle cell children compared to the control subjects ($P < 0.05$). Also, CAT activity shows significant decrease in sickle cell children compared to the control subjects ($P < 0.05$). GPx activity shows significant decrease in sickle cell children when compared to the control subjects ($P < 0.05$), while the Vitamin C and Vitamin E levels were significantly reduced in sickle cell children when compared to the control subjects ($P < 0.05$). The mean age of sickle cell children compared to the normal hemoglobin carriers ($P > 0.05$) were not statistically different.

Table 2 shows the activities of superoxide dismutase, catalase glutathione peroxidase, vitamin C, vitamin E in sickle cell teenagers and non-sickle cell teenagers. There was significant decrease in SOD activity in sickle cell teenagers compared to the control subjects ($P < 0.05$). CAT activity shows significant decrease in sickle cell teenagers compared to the control subjects ($P < 0.05$). GPx activity shows significant decrease in sickle cell teenagers when compared to the control subjects ($P < 0.05$), while the Vitamin C and Vitamin E levels were significantly reduced in sickle cell teenagers when compared to the control subjects ($P < 0.05$). The mean age of sickle cell teenagers were not statistically different from normal hemoglobin teenagers ($P > 0.05$).

Table 3 shows the activities of superoxide dismutase, catalase glutathione peroxidase, vitamin C, vitamin E in sickle cell adults and non-sickle cell adults. There was significant decrease in SOD activity in sickle cell adults compared to the control subjects ($P < 0.05$). CAT activity shows significant decrease in sickle cell adults compared to the control subjects ($P < 0.05$). GPx activity shows significant decrease in sickle cell adults when compared to the control subjects ($P < 0.05$), while the Vitamin C and Vitamin E levels were significantly reduced in sickle cell adults when compared to the control subjects ($P < 0.05$). The mean age of sickle cell adults were not statistically different from normal hemoglobin adults ($P > 0.05$).

Table 1: Parameters showing the ages, the activities of superoxide dismutase, catalase, glutathione peroxidase, vitamin C, vitamin E in non-sickle cell children and sickle cell children.

Parameters	AA	AS	SS
SOD(U/L)	141.09±2.1 ^a	138.01±2.0 ^a	120.14±2.2 ^b
CAT (U/L)	11.99±1.8 ^a	11.03±1.9 ^a	8.21±1.8 ^b
GPx (U/L)	137.05±2.2 ^a	1.36±2.1 ^a	121.06±2.0 ^b
Vit C (mg/L)	8.90±0.75 ^a	8.01±0.81 ^a	6.02±0.80 ^b
Vit E (Mmol/L)	0.72±0.02 ^a	0.71±0.03 ^a	0.51±0.02 ^b
Age(kg)	3.51±2.0 ^a	3.62±2.1 ^a	3.59±2.0 ^a

Table 2: Parameters showing the ages, the activities of superoxide dismutase, catalase, glutathione peroxidase, vitamin C, vitamin E in non-sickle cell teenagers and sickle cell teenagers.

Parameters	AA	AS	SS
SOD (U/L)	159.00±2.2 ^a	148.00±2.1 ^a	125.00±2.2 ^b
CAT (U/L)	11.01±2.0 ^a	11.00±2.1 ^a	7.01±2.1 ^b
GPx (U/L)	141.00±1.8 ^a	137.00±1.9 ^a	119.00±1.8 ^b
Vit C (mg/L)	7.89±0.88 ^a	7.80±0.90 ^a	5.91±0.8 ^b
Vit E (Mmol/L)	0.75±0.03 ^a	0.66±0.02 ^a	0.47±0.02 ^b
Age (yr)	13.71±2.2 ^a	14.10±2.0 ^a	13.99±2.1 ^a

Table 3: Parameters showing the ages, the activities of superoxide dismutase, catalase, glutathione peroxidase, vitamin C, vitamin E in non-sickle cell adults and sickle cell adults.

Parameters	AA	AS	SS
SOD(U/L)	197 ± 2.3 ^a	186.4±7.2 ^a	154.1±6.5 ^b
CAT (U/L)	11.69±2.5 ^a	11.05±2.2 ^a	8.45±2.3 ^b
GPx (U/L)	150.50±2.7 ^a	147.10±2.5 ^a	129.00±2.0 ^b
Vit C (mg/L)	9.90±1.3 ^a	8.88±1.2 ^a	6.90±1.2 ^b
Vit E (Mmol/L)	0.81±0.02 ^a	0.80±0.02 ^a	0.62±0.03 ^b
Age(kg)	23.40±3.0 ^a	24.10±3.1 ^a	23.29±3.0 ^a

IV. DISCUSSION

SCD is a hereditary disorder with higher potential for oxidative damage due to chronic redox imbalance in red cells that often results in clinical manifestation of mild-to severe hemolysis in patients with this genetic disorder (Shan et al, 1999). SS patients produces greater quantities of free radicals; O_2^- , H_2O_2 and OH^- than AA and AS patients with normal red blood cells (Nan fack et al, 2014). This present study showed a significant reduction in the activities of Superoxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and levels of Vitamin C and Vitamin E in sickle cell anemia patients (SS) compared to homozygous normal hemoglobin (AA) and heterozygous normal hemoglobin (AS) subjects across three groups (Children, Teenagers, and Adults). The reduced activities of SOD, CAT and GPx in sickled cell subjects is in accordance with the studies of Al sultan et al, 2010, Gizi et al, 2011, and Oyeyemi et al, 2016. These results suggest that HbS-containing red cells auto-oxidize faster, generating a greater extent of superoxide, hydrogen peroxide hydroxyl radicals and lipid oxidation products resulting in oxidative stress in sickle cell carriers that leads to consumption or inactivation of these antioxidants which may be related to the severity of oxidative stress in sickle cell subjects. (Steinberg et al, 2008). The reduced levels of Vitamin E in sickled cell as observed in this study is supported by the findings of Bhoi et al, 2014. The antioxidant action of Vitamin E is effective even at high oxygen concentration and thus it is concentrated in those lipid structures which are exposed to higher partial pressure of oxygen for example membranes of erythrocyte, respiratory tract & retina. Thus, decreased vitamin E level in sickle cell anaemia patients might behave as a cause or an effect to increased haemolysis (Bhoi et al, 2014)

Vitamin C, another anti-oxidant was also decreased significantly in Sickle cell subjects in all the three age groups when compared to control group which was also observed in the study of Jyoti Titus in 2004. The lowered vitamin C level in sickle cell anaemia patients indicates their exhausted status in an attempt to quench increased free radicals. Vitamin C, an aqueous phase antioxidant, has excellent protective role in regeneration of the reduced form of other powerful antioxidants namely glutathione peroxide and Vitamin E and thus stops free radical chain reaction. (Chiu et al, 2001). But when depleted, its protective role further aggravates the

complications associated with sickle cell hemoglobin. The mean age value across the three groups; in sickle cell patient did not affect the antioxidant status. This mean that enzymatic and non-enzymatic antioxidants are implicated in sickle cell anaemia irrespective of the age of the carrier.

V. CONCLUSION

SCA is associated with alterations in markers of oxidative stress, decreased antioxidant enzyme level. Also, age of the subjects appeared to play no role in their oxidative status. Thus, antioxidants could be used as effective therapeutic agents for the treatment of this disease and supplementation of patients with diets rich in antioxidants may reduce the complications of this disease in all ages.

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