



Evaluation of Antioxidant Enzymes in Children With Sickle Cell Anaemia in Ekiti State, Nigeria

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ABSTRACT: This study was carried out to determine the activities of Catalase (CAT) and Superoxide dismutase (SOD) in children with sickle cell anemia in Ekiti State, Nigeria. Sickle celled children (n=120) and control healthy children (n=80) subjects between the ages of 1 – 8 years old from the Ekiti State University Teaching Hospital, Ado-Ekiti and Federal Medical center, Ido-Ekiti, Ekiti State, Nigeria were used for the study. Plasma CAT and SOD activities were assessed in the collected blood samples. The result obtained shows that there was significant decrease in SOD and CAT activities in sickle cell subjects compared to the control subjects ($P<0.05$). There was also significant decrease in the weight and plasma pH of sickle cell subjects compared to the control subjects ($P<0.05$). Therefore, it could be concluded that antioxidant parameters, SOD and CAT activities is significantly affected or implicated in sickle cell anemia patients.

Keywords: Catalase (CAT), Superoxide dismutase (SOD), Sickle cell anemia.

I. INTRODUCTION

Sickle cell disease (SCD) is also known as sickle cell anemia (SCA), is a hereditary blood disorder characterized by an abnormality in the oxygen carrying haemoglobin molecule in red blood cells. This leads to a propensity for the cells to assume an abnormal, rigid, sickle cell like shape under certain circumstances. The severities of the complications that occur with this disorder are widely variable, but overall mortality is increased and life expectancy decreased when compared to the general population (Kathleen and Julie, 2011).

Sickle cell disease is associated with a number of acute and chronic health problem such as severe infections, attacks of severe pain (Sickle cell crisis) and stroke (Weatherall and Clegg, 2001). The overall incidence of sickle cell disease exceeds that of most other serious genetic disorders, including cystic fibrosis and hemophilia (American Academy of Pediatrics, 2002). This occurs worldwide but occurs most frequently in Africans and less commonly in those of Mediterranean, Latino, East Indian, and Arab descent (Kathleen and Julie, 2011). It is estimated that 16% of the population in Africa has a sickle hemoglobinopathy which is the highest proportion worldwide. The Americas and the East Mediterranean region represent the next highest proportion of sickle cell hemoglobinopathy as delineated by the World Health Organization (Angastiniotis and Modell, 1998).

As of 2013 about 3.2 million people have sickle-cell disease while an additional 43 million have sickle-cell trait (Global Burden of Disease Study, 2013). With regard to children with SCD in the developed world, the mortality rate was estimated to be as low as 0.5 – 1.0 per 100,000 children. This is in contrast to higher rates in developing countries such as the Republic of Benin which reported a mortality rate of 15.5 per 1000 children (or 1,550 per 100,000 children) (Rahimy *et al.*, 2009).

Sickle cell disease is a potentially devastating condition that is caused by an autosomal recessive inherited hemoglobinopathy which results in the vaso-occlusive phenomena and hemolysis (Kathleen and Julie, 2011). It is characterized by recurrent acute severe pain episodes due to vaso-occlusive crisis (VOC). The genetic abnormality is due to a substitution of amino acid valine for glutamic acid at the 6th position on the β -globin chain and was first discovered over one hundred years ago (Herrick, 1910). Haemoglobin S (HBS), the haemoglobin that is produced as a result of this defect is a haemoglobin tetramer (alpha 2/beta 2) that is poorly soluble and polymerizes when deoxygenated (Bunn, 1997).

Sickle cell diseased children have a chronic inflammatory condition that often result in low local oxygen tension leading to sickling of erythrocytes, increased blood viscosity, thrombosis with subsequent ischemic tissue break down (Hunt and Ingram, 1958). Children with SCD are also susceptible to recurrent infection, as well as increased leukocytes count even during the steady state. This elevated leukocytes counts may be due to the clogging of the spleen with sickled erythrocytes (Castro and Gladwin, 2005). The combined effect of the inflammatory response and infections in SCD produce secondary disease states such as acute chest syndrome (ACS), pulmonary hypertension (PHT) and indirectly stroke (Castro and Gladwin, 2005). Some clinical features include; anemia, severe pain, chest pain, pallor, strokes joint pain and severe infections. Sickle cell disease (SCD) children have impaired immune response and are uniquely vulnerable to infections such as malaria which is a common trigger of vaso-occlusive crisis in patients living in malaria endemic countries (Nagababu *et al.*, 2008). The most common causes of death in childhood from sickle cell diseases are infection, acute chest syndrome and stroke (Quinn *et al.*, 2004).

The care of patients with sickle cell disease is largely supportive with hydroxyurea representing the only widely used drug which modifies disease pathogenesis. Painful vaso-occlusive events are the most common complication experienced by both children and adults with sickle cell disease and there are few treatment options to prevent the development of these events (Kathleen and Julie, 2011).

Antioxidant enzyme plays an important role against oxidative stress by enzymatic neutralization of hydrogen peroxide to oxygen and water (Tiwari *et al.*, 2013). Catalase is a common enzyme found in nearly all living organism exposed to oxygen (such as vegetables, fruits or animals). It catalyses the decomposition of hydrogen peroxide to water and oxygen. (Chelikani and Lowen, 2004). It is a tetramer of four polypeptide chains, each over 500 amino acids long which contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. (Boone *et al.*, 2007). Catalase is an important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS) likewise, it is one of the highest turnover numbers of all enzymes; one catalase molecule can convert approximately 5 million molecules (Toner *et al.*, 2007) of hydrogen peroxide to water and oxygen each second. The optimum pH for human catalase is approximately 7 (Toner *et al.*, 2007).

Superoxide dismutase (SOD) is an antioxidant enzyme that catalyses the dismutation of superoxide anions into hydrogen peroxide and molecular oxygen. SOD plays important protective roles against cellular and histological damages that are produced by ROS (Tiwari *et al.*, 2013). Superoxide reacts rapidly with nitric oxide (NO), reducing NO bioactivity and producing the oxidative peroxynitrite radical (Tiwari *et al.*, 2013).

II. MATERIALS AND METHOD

Selection Of Subject

Children diagnosed of sickle cell anemia at Ekiti State University Teaching, Ado Ekiti State and Federal Medical Centre, Ido Ekiti were used as the subject for this study. A total of 120 sickle celled children were used between the ages 1– 8 years old. The subjects used for the control were 80 healthy children, age less than 8 years old. They were tested and confirmed non-sickle cell patients. The subjects' age and weight were also measured and recorded. A total of 200 subjects were used for this research work.

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. It was carefully dispensed into a fluoride oxalate bottle to avoid blood clotting. The blood plasma of the children was used as samples.

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).

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).

Result And Discussion

Table 1 shows the activities of superoxide dismutase and catalase in sickle cell children and non-sickle cell children. There was significant decrease in SOD activity in sickle cell subjects compared to the control subjects ($P<0.05$). Also, CAT activity shows significant decrease in sickle cell subjects compared to the control subjects ($P<0.05$).

The weight of abnormal hemoglobin carriers compared to the normal hemoglobin carriers ($P<0.05$) decreased significantly. The plasma pH of sickle cell subjects compared to the control subjects ($P<0.05$) decreased significantly.

Table 1: Parameters showing the activities of superoxide dismutase and catalase in non-sickle cell children and sickle cell children

Parameters	AA	AS	SS
Superoxide dismutase(U/L)	149±2.4 ^a	143±2.1 ^a	124±2.1 ^b
Catalase (U/g)	12.4±1.9 ^a	11.9±2.0 ^a	8.9±2.0 ^b
Age(yrs)	3.70±2.0 ^a	3.85±2.1 ^a	3.85±2.1 ^a
Weight (kg)	18.10±1.6 ^a	13.91±2.0 ^b	13.91±2.0 ^b
pH	9.81±0.15 ^{ab}	7.71±0.06 ^a	7.71±0.06 ^a

Results are expressed as mean and standard deviation. $P\leq 0.05$, superscript a and b indicates significant differences.

AA – Homozygous normal red blood cell

AS – Heterozygous normal red blood cell

SS – Sickle cell disease

III. DISCUSSION

The present result evaluated the activity of Superoxide dismutase (SOD), Catalase (CAT) of sickle cell anemia patients (SS) compared to homozygous normal hemoglobin (AA) and heterozygous normal hemoglobin (AS) subjects. Antioxidant enzyme plays an important role against oxidative stress by enzymatic neutralization of hydrogen peroxide to oxygen and water (Tiwari *et al.*, 2013). SOD and CAT activities in sickle cell patients (SS) decreased significantly compared to normal hemoglobin subjects. These corroborates with earlier study of Lamia *et al.* (2015). CAT activity in sickle cell (SS) patient decreased significantly compare to the normal hemoglobin subjects, this is in accordance with the study of Lamia *et al.* (2015).

The mean age value for both normal hemoglobin carrier (AA) and sickle cell patient was insignificantly difference. The weight of sickle children reduced significantly, which indicates that this hemoglobin type affects their weights. This is in agreement with the findings of Mukherjee and Gangakhedkar (2004), who stated that children with sickle cell disease weigh less, are shorter and undernourished as compared to normal children.

There was significant decrease in the plasma pH in sickle cell patients (SS) compared to normal hemoglobin (AA) individuals. This is in alignment to the findings of Bookchin *et al.* (1976), who suggested this has effect on specific ionic interactions involved in Haemoglobin gelation or possible clinical counterparts which are acute metabolic acidosis and alkalosis where the Bohr effect and oxygen affinity-independent effects of pH alterations on sickling would be additive.

IV. CONCLUSION

In conclusion, the result of the research study carried out revealed that the antioxidant enzymes (SOD and CAT) activities significantly decreased in the sickle cell children compared to the normal hemoglobin subjects. It can therefore be concluded that antioxidant indices (SOD and CAT) are affected by the types of hemoglobin inherited by individuals. Hence children with SS can be advised to be fed more with diet that can aid the production of these antioxidants.

Also, the weight of sickle cell children reduced significantly. This work therefore supports the growing evidence that Sickle cell anemia patients are subjected to chronic oxidative stress which is able to cause oxidative damage in biological macromolecules. It is therefore recommended that the place of antioxidant should not be overlooked in the management of sickle cell anemia in children.

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