

Full Length Research Paper

A study of the antioxidant vitamin E in blood sample of steady state sickle cell anaemia and non-sickle cell anaemia patients

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Sickle cell anaemia is an inherited disorder of haemoglobin characterized by sickled red blood cells and increased destruction of these cells. This research aims to study the antioxidant vitamin E in blood sample of steady state sickle cell anaemia patients and that of non-sickle cell anaemia in Maiduguri, Borno State, North-eastern Nigeria. Sickle-cell anaemia is a hereditary disorder, which results into various types of crisis such as chronic acute pain syndromes, severe bacterial infections, and necrosis (tissue death), although, there is no cure for sickle cell disease. However, with a nutritionally balanced diet, other supplements such as vitamins E, A and C, well hydration and taking care of infection potentially help lessen the effects of the disorder. Vitamin E improves vascular endothelial vasodilatory function by inhibitory effects on lipid peroxidation or by protein kinase activation or by enhancement of nitric oxide (NO) dependent mechanism. It also increases femoral blood flow (FBF) and decreased forearm vascular resistance in sickle cell anaemia (SCA) patients. This study was carried out on steady state sickle cell anaemia patients attending the paediatrics and haematology clinics of the University of Maiduguri Teaching Hospital (UMTH) as a referral center. Undergraduate students, secondary, primary and pre-primary school students of the University of Maiduguri, Borno State, were incorporated in the study as controls. A total number of 120 subjects were enrolled into the study constituting 60 subjects with homozygous SS, and 60 controls who are homozygous AA. High performance liquid chromatography (HPLC) was used for the determination of the vitamin E. The mean vitamin E in SCA in relation to age, occupation, educational level, and BMI were 0.072 ± 0.004 mg/ml, 0.059 ± 0.001 mg/ml, 0.071 ± 0.001 mg/ml, 0.70 ± 0.001 mg/ml respectively and that of the control were 0.092 ± 0.001 mg/ml, 0.081 ± 0.001 mg/ml, 0.092 ± 0.001 mg/ml, and 0.103 ± 0.001 mg/ml. These values were significantly lower ($P < 0.05$) in SCA patients compared to that of the control. In conclusion, there is a decrease in vitamin E levels in SCA patients in the steady state with controls. These results also showed that the level of education improved the vitamin level of the SCA patients.

Key words: Sickle cell anaemia, vitamin E, age, level of education.

INTRODUCTION

The term sickle cell disease (SCD) is used in a generic sense to refer to all the clinically severe haemolytic syndromes (Desai and Dhanani, 2004). Sickle cell anaemia results from the substitution of a valine residue for glutamic acid at position 6 in the beta subunit of

haemoglobin (Ingram, 1956). People with only one gene for HbS are phenotypically normal (sickle cell trait), while those who inherited two HbS genes from their parents have SCA. The major clinical features of SCA are acute episodes of pain, stroke, priapism and acute chest syndrome and chronic organ damage, for example, osteonecrosis, renal failure and chronic haemolytic anaemia (Ohene-Frempong et al., 2001).

Free radicals and antioxidants are generated as part of

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normal aerobic cellular existence (Jaja et al., 2003). However, the generation of excess free radicals has been implicated in the pathophysiology of conditions like SCA, rheumatoid arthritis, arterosclerosis, retinopathies, diabetes mellitus and aging (Chiu et al., 1982; Sen, 1995). The results of the generation of free radicals and reactive oxygen species (ROS) are lipid peroxidation (Jaja et al., 2003). Damage to membranes by free radicals leads to compromised integrity of the membrane fluidity and inactivation of membrane bound receptors and enzymes (Hallwel and Gutterdge, 1989). Apart from interacting with the bilayer structure of the cell, free radicals also degrade proteins and promote DNA-stranded breakage thus causing damage to other genomic structures (Clarkson and Thompson, 2000) and thus might play a significant role in the pathophysiology of SCA related microvascular dysfunction, vaso-occlusion and development of organ damage (Wood and Granger, 2007).

Vitamin E is a fat soluble vitamin that exists in eight different forms (alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol) that have varying levels of biological activity. Alpha tocopherol (α -tocopherol) is the most active form of vitamin E in humans. It is also a powerful biological antioxidant (Traber, 1999). The use of antioxidants such as vitamin E *in-vitro* seems to reduce the generation of pro-oxidants which could reduce cell membrane damage, adhesion and phagocytosis of oxidized erythrocytes, consequently reducing haemolysis (Amer et al., 2008; Fibach and Rachmilewitz, 2008; Nur et al., 2011). Therefore dietary counselling is an important part of routine health care of children with SCA.

The aim of this study is to determine the antioxidant vitamin E in steady state sickle cell anaemia patients and that of non-sickle cell anaemia individuals in Maiduguri metropolis.

METHODOLOGY

A total number of 120 subjects were enrolled into the study constituting 60 subjects with sickle cell anaemia (homozygous with SS) who are in the steady state. The term steady state is a misnomer, being characterized by biochemical and rheological fluctuation consistent with minor episodes of microvascular occlusion that are insufficient to cause the overt tissue infarction of painful crisis (Akinola et al., 1992), and 60 controls who are homozygous AA, comprising both sexes (males and females). The age group range is from 1.3 to 35 years. A cross-sectional study was carried out at the University of Maiduguri Teaching Hospital (UMTH) that serves as a referral center, while undergraduate students, secondary, primary and pre-primary school students of the University of Maiduguri, Borno State were taken as the control group. About 5 ml of blood samples were withdrawn from each subject and the control aseptically for the

determination of antioxidant vitamin E. In the subjects, in the case of the control, the genotype was also determined to ensure that the haemoglobin genotype was AA.

The sickle cell subjects were those that attended the sickle cell haematology/paediatric clinic that were at their steady state. Random sampling technique was employed in the selection of the control groups who were undergraduates, secondary primary and pre-primary school of the University of Maiduguri, whose parents/guardians agreed with the informed consent. Informed consent was obtained from the subjects before the administration of questionnaire. Ethical clearance was obtained from University of Maiduguri Teaching Hospital Maiduguri, Borno State. Subjects that were excluded are those who have been transfused in the last three months, or had ill health, or in crisis, or have not given any consent.

The blood samples were protected from sunlight. Specimens were taken to the National Drug and Food Administration and Control (NAFDAC) Area laboratory for analysis. Haemoglobin electrophoresis was carried out on all control subjects using the lactate cellulose method (Dacie and Lewis, 2006), at the UMTH haematology laboratory for the determination of haemoglobin genotype. High performance liquid chromatography (HPLC) was used for the determination of vitamin E.

Preparation of standard vitamin E stock solution

An ample of vitamin E containing 1000 mg was used. It was then diluted with 50 ml of acetonitrile. About 4 ml of the above stock was pipetted and diluted with another 50 ml of acetonitrile. This represents the final dilution for vitamin E. The above stock solution was made into 100 ml of acetonitrile.

High performance liquid chromatography is basically a highly improved form of column chromatography. Blood samples were spinned at 5,000 rpm for 5 min to get the serum. Serum was separated and transferred into a 5 ml plain bottle, protected from sunlight. About 1 ml of the serum was withdrawn into 5 ml of plain bottle and 2 ml of acetonitrile was added to precipitate the proteins. The solution was allowed to settle for 2 min and the clear solution was filtered with a 0.2 μ m acrodisc to remove any remaining particle. 1 ml of the filtrate was then mixed with 4 ml of the final stock solution containing 0.0769 mg/ml of vitamin E freshly prepared. The solution was thoroughly mixed and transferred into a matrix vial that was placed into the matrix of the HPLC machine for analysis. The HPLC machine was calibrated at a wave length of 280 nm for 10 min. The appearance of the vitamin E peak appeared at 6.9 min. The timing for the determination of the vitamin is based on initial observation using the standards of the vitamin; this is the timing that the vitamin E appeared.

The results obtained were a combination of the

Table 1. Mean (\pm SEM) level of vitamin E (mg/ml) in steady state SCA patients and control at different age groups.

Age group (in years)	Mean vitamin A (mg/ml) \pm SEM	
	Control (n=60)	SCA (n=60)
0-5	0.078 \pm 0.006	0.068 \pm 0.005
6-10	0.083 \pm 0.002	0.073 \pm 0.002
11-15	0.082 \pm 0.001	0.067 \pm 0.004*
16-20	0.074 \pm 0.002	0.057 \pm 0.002*
21-25	0.070 \pm 0.005	0.068 \pm 0.003
26-30	0.091 \pm 0.001	0.082 \pm 0.003
31-35	0.095 \pm 0.001	0.069 \pm 0.006*

* Significant relative to control, *P < 0.05, Z-test.

standard as well as the vitamin present in the sample. The vitamin E level was calculated using the following formula:

% vitamin content in a sample = peak area of sample/peak area of standard \times 100.

Concentration of vitamin in a sample = %content/100 \times concentration of standard in the stock (vitamin E, 0.0769 mg).

The above formula gave the total amount of the concentration of both the standard and that of the sample. To obtain the quantity of vitamin E in the sample alone, the concentration of the standard in the stock is subtracted from the concentration calculated by the methods of Nierenberg and Lester (1985) and Mario et al. (1999), and the standard method for drug determination at NAFDAC.

The data were collected and collated into a Statistical Package for Social Sciences (SPSS) version 16 for the analysis of the various parameters. The mean, standard deviation, was obtained and Z-test was used to obtain relationship between individual parameters in relation to experimental and control groups. The results obtained are presented in tables. However, values less than 0.05 were considered significant and values greater than 0.05 were considered insignificant at a confidence level of 95%.

RESULTS

The mean serum vitamin E serum level in sickle cell patients in the steady state ranged between 0.057 \pm 0.002 and 0.082 \pm 0.003 mg/ml, while that of the control ranged between 0.070 \pm 0.002 and 0.095 \pm 0.001 mg/ml (Table 1). The mean minimum and maximum of vitamin E level of the subjects were significantly lower (P<0.05) than that of the control. The result of mean vitamin E in SCA patients indicated that the age group of 16-20 years had the lowest level (0.057 \pm 0.002 mg/ml), while the

maximum level (0.082 \pm 0.003 mg/ml) was found in the age group of 26-30 years. Whereas, the control group had the minimum at the age group of 21-25 years (0.070 \pm 0.005 mg/ml) and the maximum (0.095 mg/ml) in the age group of 31-35 years.

The mean level of serum vitamin E in SCA patients based on their occupational status was presented in Figure 1. The minimum mean vitamin E level was found in the unemployed (0.061 \pm 0.007 mg/ml), while the maximum (0.074 \pm 0.004 mg/ml, 0.071 \pm 0.004 mg/ml) was observed in the student and business class respectively.

Serum vitamin E level was evaluated based on the educational status of both sickle cell at the steady state and non-sickle cell (Figure 2). Serum minimum vitamin E level in the SCA patients and the control recorded in the pre-primary level was 0.063 \pm 0.001 mg/ml and 0.075 \pm 0.009 mg/ml, respectively. Maximum vitamin E level in the serum of both SCA patients and control from the tertiary level was 0.078 \pm 0.001 mg/ml and 0.106 \pm 0.009 mg/ml respectively. The minimum and maximum levels of vitamin E were both significantly higher (P< 0.05) in control than the minimum and maximum levels of vitamin E serum in SCA patients.

The results of this study showed that in SCA patients, only underweight and normal weight were recorded, but in the control group, overweight and obese were also recorded. Serum vitamin E level in normal weighted of SCA subjects recorded was 0.066 \pm 0.004 mg/ml while in underweight SCA patients the value was 0.073 \pm 0.003 mg/ml.

The difference between these values was not significant (P>0.05). In this study, it was found that vitamin E serum level in the control group with normal body weight and obese was 0.103 \pm 0.009 mg/ml and 0.143 \pm 0.001 mg/ml, respectively (Table 2). Vitamin E level was found to be significantly higher (P<0.05) in the control with normal weight (0.103 \pm 0.009 mg/ml) and underweight (0.085 \pm 0.002 mg/ml) when compared with SCA patients with normal weight (0.066 \pm 0.004 mg/ml) and underweight (0.073 \pm 0.002 mg/ml), respectively.

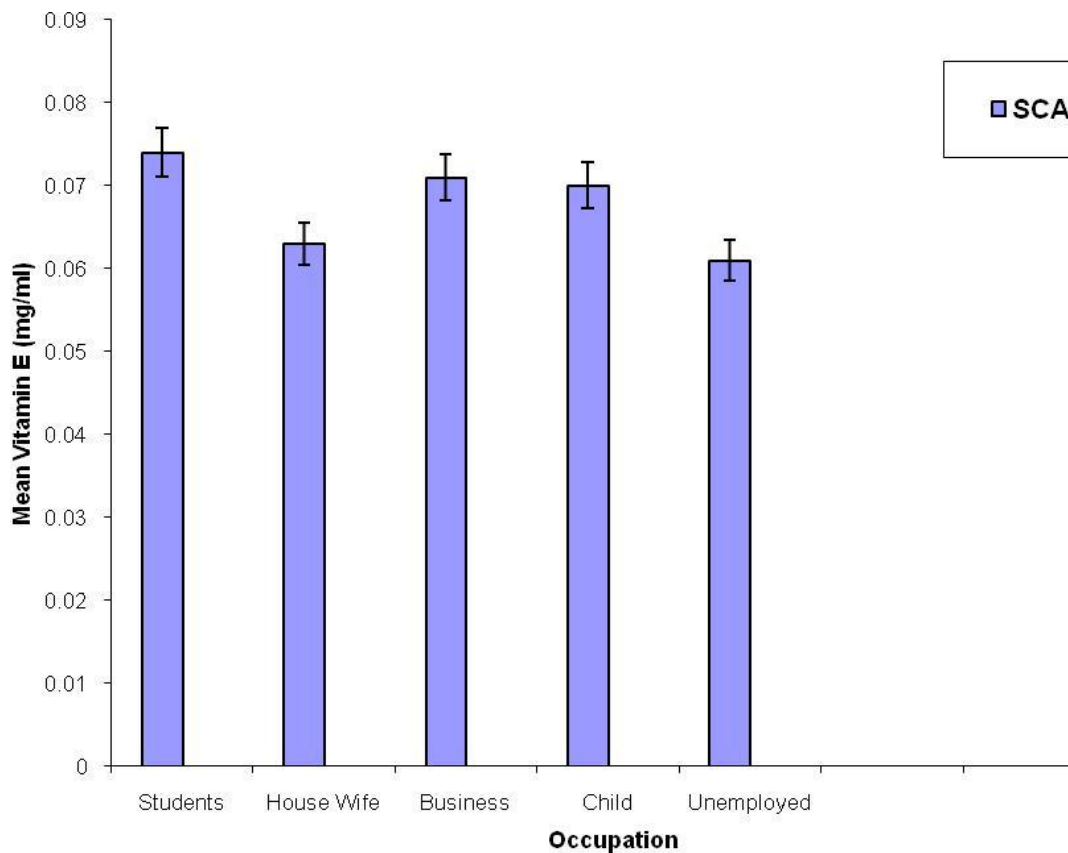


Figure 1. Mean (\pm SEM) level of Vitamin E (mg/ml) in steady state SCA patients according to occupation.

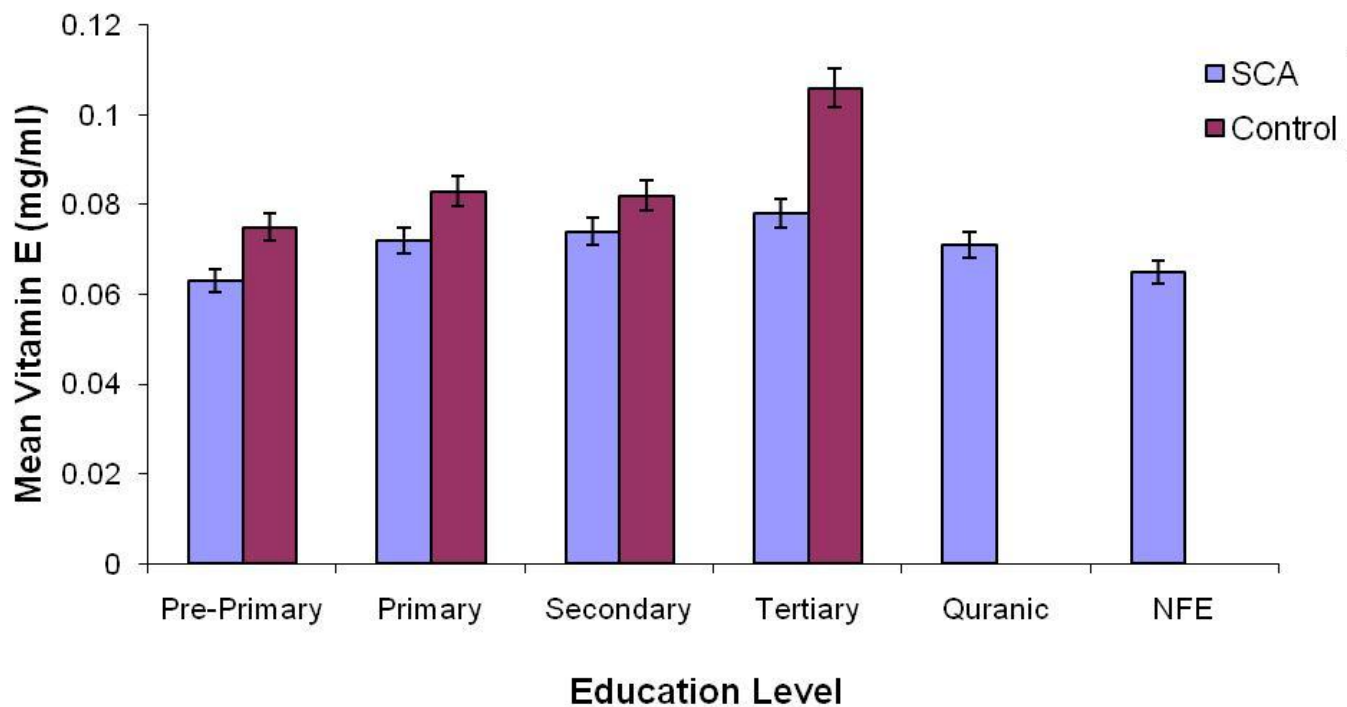


Figure 2. Mean \pm SEM vitamin E level (mg/ml) in steady state SCA patients according to educational level and that of the control.

Table 2. Mean (\pm SEM) level of vitamin E (mg/ml) in steady state SCA patients and control according to BMI.

BMI	Mean \pm SEM (mg/ml) of Vitamin A	
	Control (n=60)	SCA (n=60)
Underweight	0.085 \pm 0.003	0.073 \pm 0.003*
Normal weight	0.103 \pm 0.005	0.066 \pm 0.004**
Overweight	0.082 \pm 0.001	-
Obese	0.143 \pm 0.001	-

* Significant relative to control, *P < 0.05, Z-test.

DISCUSSION

In homozygous sickle cell anaemia, decreased serum vitamin E is present. It was also found that vitamin E type antioxidant capacity was significantly lower in SCA patients than in non-SCA (age and sex) matched controls.

Antioxidants are potentially protective agents that may help guard against oxidative haemolysis (Attakon et al., 2006). Vitamin E is considered the most important lipid-soluble exogenous antioxidant in humans and it increases plasma vitamin E level and reduces plasma oxidants (Margaristus et al., 2003; Unchern et al., 2003). In this study, the level of serum vitamin E in both SCA and the control group was investigated. It was found that the level of vitamin E in SCA steady state was lower than that of the control. This study is in agreement with the earlier reports that vitamin E was lower in SCA patients (Taippe et al., 1961; Natta and Machlin, 1979; Natta et al., 1988; Sindel et al., 1990; Hasnato, 2006; Ray et al., 2007). This low plasma vitamin E level in SCA patients has been related to the elevated level of irreversibly sickle cells (Ndombi and Kinoti, 1990). This finding was also supported by Jaja et al. (2005) who indicated that supplementation with γ -tocopherol decreased the percentage of irreversible sickled cells and increased foetal haemoglobin concentration. High level of irreversible sickled cells will increase intravascular haemolysis of red cell membrane and therefore accumulation of haemoglobin in the plasma which is known to be a potent catalyst of lipid oxidation. The plasma haemoglobin might therefore catalyze the oxidative destruction of tocopherol and account for the lower plasma tocopherol levels in these subjects. Adelekan et al. (1989) also reported that there was reduced antioxidant capacity in paediatric and in adult patients with homozygous SCA. Vitamin E acts to protect cells against the effects of free radicals which are potentially damaging by-products of energy metabolism.

Patients with sickle cell anaemia have a potential for oxidative damage due to the chronic redox imbalance in red blood cells that often leads to haemolysis, endothelial injury and recurrent vaso-occlusive episodes. The oxidative status of the cell is determined by the balance

between the pro-oxidant and antioxidants. Pro-oxidants referred to as reactive oxygen species (ROS) are classified into radicals and non-radicals. The radicals are highly reactive due to their tendency to accept or donate an electron and attain stability. When cells experience oxidative stress, ROS, which are generated in excess, may oxidize proteins, lipids and DNA leading to cell death and organ damage. Oxidative stress is believed to aggravate the symptoms of many diseases, including haemolytic anaemia. Oxidative stress was found in β -haemoglobinopathy (SCA and thalassaemia).

In this study, vitamin E level in different age groups was also investigated. It was observed that the serum vitamin E level was highest in the age group of 26-30 years in SCA subjects (0.082 mg/ml) and the control group had the highest in the age group of 31-35 years (0.095 mg/ml). The mean vitamin E level for the subjects and the control was 0.071 and 0.092 mg/ml, respectively. This showed that the normal subjects had a higher vitamin E level than the SCA patients, and this may explain the reason why sickled red cells are susceptible to haemolysis as the protective function by vitamin E is low.

A comparative analysis was also carried out to determine the vitamin E level based on occupational status of SCA in steady state. It was observed that the highest level of vitamin E was from the students and business class category (0.074 and 0.071 mg/ml respectively), and the lower level was observed in the unemployed category (0.061 mg/ml). This might be due to the better socio-economic status and educational level in this category compared to the other groups who might not have enough means to support their ailment.

Evaluation of vitamin E level in SCA in their steady state with respect to educational status indicated that the level of education significantly increased the level of vitamin E, though the value obtained in the control group was higher than the SCA patients. This result indicated that the level of education may play a significant role in the health status of an individual.

When vitamin E level was analyzed according to BMI, it was found out that SCA subjects were either underweight or normal weight, while overweight and obese were not recorded when compared to the control group. The probable reason for the decrease in BMI might be as a result of the many complications, including growth retardation (decreased height and weight compared to their peers), chronic hemolytic anemia, recurrent and painful vaso-occlusive episodes, acute chest syndrome and impaired immune function. Although the exact reasons were not well established, the literature indicated that low levels of zinc, folic acid, and vitamins A, C and E could be contributing factors (Powers, 1975; El-Hazim, 1979; Finan et al., 1988; Pelligrini et al., 1995; Williams et al., 1997; Leonard et al., 1998). The present study was also in agreement with the work of Chijioko (2009), who observed that the SCA patients who were underweight was about 92.5%, and only 5.7% were overweight. The

worker also reported that lower BMI in SCA subjects was a reflection of the severity of the disease and the quality of care available to them.

Conclusion

This study showed that the antioxidant vitamin E is found to be lower in the SCA subjects compared to the normal control in all age groups. The level of education may play some role in the level of antioxidant in those subjects in the tertiary institution, in having higher vitamin E levels than others. The low BMI of the SCA patients noted in this study may be as a result of the disease entity and its manifestations.

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