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Vitamin A Status of Steady State Sickle Cell Anaemia Patients Compared to Normal Control in Maiduguri North Eastern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. This work was carried out in collaboration with author MAT performed the lab work, wrote the protocol, the first draft of the manuscript and presented the work. Author SOO designed the work. Author JPA managed the literature searches and protocol author HAS supervised the work author OE performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Sickle cell anaemia is an inherited disorder of haemoglobin characterized by sickled red blood cells and increased destruction of these cells. Antioxidants protect cells from the damaging effects of free radicals. The aim of this study was to determine antioxidant vitamin A in steady state sickle cell anaemia patients and that of controls in Maiduguri, Borno state North-Eastern Nigeria. The study was carried out at UMTH Maiduguri. Sixty sickle cell anaemia patients were compared with sixty controls, aged ranged 1 year 3 months to 33 years of age, using HPLC for vitamin A status. The mean vitamin A in sickle cell patients according to age ranged between 0.047±0.002 to 0.053±0.002 mg/ml, while that of controls is 0.053± 0.001 to 0.091±0.001 mg/ml. The maximum mean serum vitamin A (0.053±0.001 mg/ml), in SCA was found in the business and children groups while the minimum vitamin A (0.039±0.001 mg/ml) was recorded in the un-employed SCA

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patients. The study showed the antioxidant vitamin A was found to be lower in the SCA subjects than in normal control in all age groups. Level of education also plays a role in the level of antioxidant vitamins in the blood.

Keywords: Sickle cell anaemia; vitamin A status; age and level of education.

1. INTRODUCTION

Sickle cell anaemia is an inherited disorder of haemoglobin that is characterized by sickled shape red blood cells (RBC) which are prone to an increased destruction. The incidence is more common in black races and sub-saharan Africa. Sickle cell anaemia results from the substitution of a valine residue for glutamic acid at position six of the beta subunit of haemoglobin [1].

In Nigeria sickle cell genes is fairly and evenly distributed with carrier rate of about 25% in the south, and between 18-25% in the north. The highest frequencies have been recorded among the Kanuries (27.9%) of Borno State, Bades (32.6%) of Yobe State and the Garkis (28.9%) of Kano State in the north. The distribution of sickle cell disease in igbos of the eastern state is 24.3%, while in the yorubas of the western region, the frequency of 23.9% [2-5]. Various studies have shown that the life expectancy is shortened in sickle cell patients with an average of 42 and 48 years for males and females respectively [6]. The studies further showed that the affected subjects are frequently deficient in a variety of micronutrients including vitamin A.

Vitamins are essential micronutrients though in very small amounts but they are very important in maintaining the fundamental functions of the body. The discovery of vitamins which includes A dates back to 19th century, indicating that other factors after carbohydrates, proteins and lipids were necessary to keep good health and also found to possess antioxidant function. Vitamin A is fat soluble retinoids [7-9] is involved in immune function, vision, reproduction and cellular communication. More so their important roles in growth, differentiation and maintenance of the heart, lungs and kidneys have been demonstrated [9]. Recent estimates by the World Health Organization (WHO) [10] showed that 231 million children in more than 90 countries are clinically or sub-clinically deficient in vitamin A [11]. This might be the cause of high incidence of child mortality and morbidity rate in those countries resulting from lack of regulatory role of vitamin A on immune system and prevention of infection through its role in formation of white blood cells.

The present study therefore, aimed at studying the normal blood level of vitamin A in sickle cell patients at steady state and normal subjects. This will provide more information as to whether to supplement vitamin A in sickle cell anaemia.

2. METHODOLOGY

A cross sectional study of sickle cell anaemia patients (HbSS) at University of Maiduguri Teaching Hospital (UMTH) was carried out. The subjects were from haematology/Paediatric sickle cell anaemia clinic. The non-sickle cell subjects (HbAA) were from University of Maiduguri pre-primary, primary, secondary and undergraduate levels. 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, and 60 controls who are homozygous AA, of both sexes (males and females) with age range between 1.3 to 35 years. The sickle cell group comprise 29 male (48.3%) and 31 females (51.66%) with age range of one year three months and 33 years, while the normal group comprised 40 males (66.6%) and 20 females (33.3%) with their age range between 3 years and 35 years.

Five ml of blood samples were withdrawn from each sickle cell subjects and control aseptically for the determination of antioxidant vitamin A. The genotype of control group was also determined to ensure the haemoglobin is AA. A random sampling technique was employed in the selection of the control group after consent was obtained from the subjects or the parent in case of children. Ethical clearance was also obtained from UMTH Maiduguri. Any subject receiving vitamin A supplement in the last two months before the clinic day was also excluded, and sickle cell patients who have been transfused in the last three months or with complains of ill or in crisis was also excluded. Plastic syringe and needles were used to obtain blood from ante-cubital vein into plain and EDTA bottles (for the determination of haemoglobin genotype in case of controls). The blood samples were protected from sunlight. Specimens were taken to the National Drug and Food Administration and

Control (NAFDAC) North-East zonal head office for laboratory analysis of vitamin A using High Performance Liquid Chromatography (HPLC) technique. Haemoglobin electrophoresis was carried out on all control subjects using the lactate cellulose method [12], at the UMTH haematology laboratory for the determination of haemoglobin genotype.

2.1 Laboratory Analyses

2.1.1 Preparation of standard vitamin A stock solution

An ample of vitamin A containing 500 mg was that of USP Rockville obtained from NAFDAC, Maiduguri. It was then diluted with 50ml of acetonitrite. Four ml of the above stock was pipetted and diluted with another 50ml of acetonitrite. This represents the final dilution for vitamin A.

2.1.2 HPLC for the determination of antioxidant vitamin A in blood sample

High performance liquid chromatography is basically a highly improved form of column chromatography. Blood samples were spinned at 5,000 R.P.M for five (5) minutes to get the serum. Serum was separated and transferred into a 5ml plain bottle, protected from sunlight. One ml of the serum was withdrawn into five ml of plain bottle and two ml of acetonitrite was added to precipitates the proteins. The solution was allowed to settle for 2 minutes and the clear solution was filtered with a 0.2 µm acrodisc to remove any remaining particle. One ml of the filtrate was then mixed with four ml of the final stock solution containing 0.0512 mg/ml of vitamin A was prepared freshly. The solution was thoroughly mixed and transferred into a matrix vial which was then placed into the matrix of the HPLC machine for analysis. The HPLC machine was calibrated at a wave length of 280nm for 10 minutes. The appearance of the Vitamin A peak was recorded at 2.6 minutes. The timing for the determination of the vitamin is based on initial observation using the standards vitamin (NAFDAC).

The results obtained were a combination of the standard as well as the vitamin present in the serum sample. The vitamin A in the serum sample was calculated using the following formula:

- % vitamin content in a sample = Peak area of sample/ Peak area of standard x 100.
- Concentration of vitamin in a sample = %content/100 x concentration of standard in the stock (vitamin A 0.0512 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 A in the sample alone: the concentration of the standard in the stock was subtracted from the concentration calculated [13-15].

The data was collected and collated into a statistical package for social sciences (SPSS) version 16 for the analysis of the various parameters. All values were expressed as the mean±SD 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. Values less than 0.05 was considered significant and values greater than 0.05 was considered insignificant at a confidence level 95%.

3. RESULTS

The mean vitamin A in sickle cell patients in the steady state according to age ranged between 0.047±0.002 to 0.053±0.002 mg/ml, while that of control ranged between 0.053±0.001 to 0.091±0.001 mg/ml. The mean vitamin A in SCA aged 21 and 25 years had the minimum level (0.047 mg/ml), while the maximum value (0.053 mg/ml) was found in the age group of 26-30 years. The control group had the minimum vitamin A value (0.053 mg/ml) at the age group between 0-5 years and the maximum value (0.091 mg/ml) in the same age group 26 and 30 years seen in the control subjects (Table 1).

The mean serum of vitamin A in SCA base on their occupational status was presented. The minimum mean Vitamin A value (0.039±0.001 mg/ml) was found in the unemployed, while the maximum value (0.053±0.002 mg/ml) was observed in the business and children groups. The mean average for the sickle cell anaemia patients was 0.049±0.001 mg/ml.

4. DISCUSSION

The outcome of the present study showed that the serum vitamin A level in SCA blood subjects with respect to age was lower than that of the normal subjects.

Table 1. Mean (±SEM) level of vitamin A (mg/ml) in steady state SCA patients and control at different age group

Age group (in years)	Mean vitamin A (mg/ml)±SEM	
	Control (n=60)	SCA (n=60)
0-5	0.053±0.001	0.051±0.004
6-10	0.058±0.001	0.051±0.002
11-15	0.069±0.001	0.049±0.004
16-20	0.057±0.002	0.049±0.002
21-25	0.078±0.005	0.047±0.002
26-30	0.091±0.001	0.053±0.002
31-35	0.065±0.001	0.048±0.001

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

Table 2. Mean Vitamin A level (mg/ml) ± SEM in steady state SCA patients according to occupation

Occupation	Mean vitamin A level (mg/ml) ± SEM SCA (n=60)
Students	0.050±0.002
House wife	0.048±0.001
Business	0.053±0.004
Child	0.053±0.002
Unemployed	0.039±0.001

The highest (0.053 mg/ml) in SCA was in the age group of 26-30 years, while the least (0.047 mg/ml) was in the age group 21-25 years when compared with the control group. However, the age group of 0-5 years had the least serum vitamin A (0.053 mg/ml ± 0.001) was recorded within age group less than five years. This finding is in consonance with the study by previous workers [16-20] that vitamin A is generally lower in SCA patients than the normal subjects. The low levels of vitamin A in SCA subjects observed in this study may be a reflection of the low immunity in SCA patients which makes them susceptible to repeated infections or at this age they are more vulnerable to malnutrition and vitamin deficiency. Also they are more prone to repeated crisis and more of the vitamins are used up. Vitamin A is commonly known as an anti-infective vitamin because it is required for normal functioning of the immune system [21]. Vitamin A also plays an important role in the development and differentiation of white blood cells (WBC). Lymphocytes plays critical roles in the immune response, activation of T-lymphocytes, the major regulatory cells of the immune system, appears to require vitamin A all-trans-RA binding RAR [21].

Vitamin A serum levels in SCA patients were also evaluated according to the occupational status. Our results showed that the business class and in children with SCA had the highest serum vitamin A level (0.053 mg/ml) than other occupational groups, the least was found in the unemployed (0.039 mg/ml). It seems that socio-economic factors have strong bearing on vitamin A level in this class of people can take good diet to support their living. Our observation is also confirmed by the presence of low levels of vitamin A in the unemployed SCA because getting adequate good diet may be a herculean task to them.

Higher values of vitamin A (0.106 mg/ml) was recorded in those group of non-sickle cell anaemia attending tertiary institution, in the normal subject it was 0.106 mg/ml, while in the SCA patients it was 0.078 mg/ml. Thus the level of education plays an important role in the maintenance of vitamin A level. This might be the reason that immunity in adults at the tertiary level is higher than those in the primary who are mainly children. The least was found in the pre-primary school children in both SCA patients and the control.

Table 3. Mean vitamin A level (mg/ml) ± SEM in steady state SCA patients and that of control according to the state of their educational level

Educational level	Mean vitamin A level (mg/ml)) ± SEM	
	Control (n=60)	SCA (n-60)
Primary	0.058±0.001	0.051±0.002
Secondary	0.062±0.001	0.049±0.002
Tertiary	0.070±0.005	0.052±0.001
Quranic	-	0.049±0.003
NFE	-	0.048±0.003
Pre-primary	0.054±0.001	0.045±0.002

*Key: NFE - No Formal Education; * Significance relative to control, *P < 0.05, Z-test*

Those in the pre-primary school are mainly under-five that naturally have low immunity and common problems of malaria, respiratory tract infection, diarrhea and vomiting, which are all related to low immunity as revealed by other study [19].

5. CONCLUSION

The results of this study showed that the antioxidant vitamin A was lower in the SCA subjects than in normal control in all age groups. It could be speculated that the lower vitamin A in SCA patients may also contribute to their inability to protect themselves from infections. And it might also play a major role in their crisis as speculated by other authors.

6. RECOMMENDATIONS

1. A wider study should be conducted to determine the level of other antioxidant vitamins amongst the subjects and the control.
2. The level of the antioxidant vitamins should be determined in rural communities to establish if there are environmental factors associated with the low antioxidant vitamins in the SCA subjects compared to the control, since the present study was done in the urban area.
3. Antioxidant vitamins A can be considered as routine drugs to all SCA patients to improve on their health status.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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