

SERUM LEVELS OF ANTIOXIDANT VITAMINS IN FOETAL HAEMOGLOBIN (HbF) PERSISTENT SICKLE CELL ANAEMIA CHILDREN IN SOKOTO, NIGERIA

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Key words: Sickle cell anaemia, foetal haemoglobin, serum vitamin A, serum vitamin C, serum vitamin E

Abstract

Background: sickle cell anaemia (SCA) is one of the commonest health problems of Nigerian children.

Method: The serum levels of antioxidant vitamins A (retinol), C (ascorbic acid) and E (alpha-tocopherol) were determined in foetal haemoglobin persistent sickle cell anaemic (Hb SS + F), sickle cell anaemic (Hb SS) and normal (Hb AA) children.

Results: The levels of antioxidant vitamins in Hb SS+F children were found to be insignificantly different ($p>0.05$) when compared to those of Sickle Cell anaemic (Hb SS) children. While significantly higher values ($p<0.05$) of the vitamins in normal children (Hb AA) were observed when compared to those in HbSS+F and Hb SS children. The slightly less depressed levels of the antioxidant vitamins in Hb SS+F children may probably be attributed to the relatively less frequent sickle- cell crises often found in these individuals.

Conclusion: The less depressed serum levels of anti-oxidant vitamins in HbSS + F children may be contributory factor to their relatively stable clinical status. Therefore, it is recommended that HbSS + F children as well as HbSS children be placed on regular anti-oxidant vitamins – A, C and E supplement.

Mots clés: Anémie drépanocytaire, hémoglobine foetale, vitamine sérique A, vitamine sérique C, vitamine sérique E

Résumé

Contexte: L'anémie drépanocytaire est l'un des plus fréquents problèmes de santé chez l'enfant au Nigeria.

Méthode : Le taux sérique d'anti oxydants, vitamine A (rétinol), vitamine C (acide ascorbique) et vitamine E (alpha-tocopherol) a été déterminé chez des enfants présentant une association hémoglobine foetale – anémie drépanocytaire persistante (Hb SS + F), une drépanocytose (Hb SS) et une hémoglobine normale (Hb AA).

Résultats : Le taux de vitamines anti oxydants n'était pas significativement différente entre les enfants présentant une Hb SS+F et ceux présentant une Hb SS ($p>0.05$). Par contre ce taux de vitamines est significativement plus élevé chez les enfants à Hb normal (AA) comparés à ceux ayant une Hb SS+F et SS ($p<0.05$). La faible baisse du taux de vitamines anti oxydants chez les enfants à Hb SS+F pourrait s'expliquer par la relative moindre fréquence des crises de drépanocytose souvent retrouvée chez ces enfants.

Conclusion: La faible baisse du taux de vitamines anti oxydants chez les enfants à Hb SS+F pourrait constituer un facteur contribuant à leur relative stabilité sur le plan clinique. Il est donc recommandé de mettre les enfants à Hb SS+F et SS sous traitement anti oxydant: A, C et E.

Introduction

Sickle cell anaemia (SCA) is one of the commonest health problems of Nigerian children that are related to a genetic blockage. It is a hereditary blood disorder caused by the substitution of valine for glutamic acid in the sixth position of the β -chain of the haemoglobin (HbS). The red blood cells (RBC) containing HbS assume irregular pointed shapes resembling sickles when exposed to low oxygen tension. Such blood cells are less efficient at carrying oxygen than normal cells and unable to pass easily through blood capillaries, with the result that blood vessels are clogged leading to impaired circulation.¹ Herrick,² first reported SCA, a chronic molecular disease of haemoglobin.¹

Haemoglobin, which is contained in the RBCs, serves as the oxygen carrier in the blood while myoglobin, which is located in the muscle, serves as a reserve supply of oxygen and facilitates the movement of oxygen within the muscle³. It also plays a vital role in the transport of carbon dioxide and hydrogen ions.⁴

During human embryonic and foetal development, an orderly change occurs in haemoglobin.⁵⁻⁷ during the second and third trimester, foetal haemoglobin ($\alpha_2\gamma_2$) becomes the dominant haemoglobin until six months after birth, when foetal haemoglobin is gradually replaced by haemoglobin A ($\alpha_2\beta_2$), the principal haemoglobin in normal individuals.⁸

Hereditary persistence of foetal haemoglobin (HPFH) refers to a situation in which the normal switch from foetal to adult haemoglobin synthesis does not occur at birth.⁹ This defect occurs as a result of a deletion of both the beta and delta globin genes with the resultant persistence of foetal haemoglobin ($\alpha_2\gamma_2$) into adult in the absence of any haemoglobin abnormality.⁹⁻¹³

In heterozygous persons, the level of HbF is 15-30%. Martin and Belter,¹⁰ using lysate levels of foetal haemoglobin and Zago et al¹¹ using immunologic techniques for counting of cells showed that foetal haemoglobin and high F-cells levels (>80%) in normal adults are inherited. When both the high foetal gene and the sickle cell genes are present in the same persons, haematological manifestations are very mild. This is because there is an even distribution of foetal haemoglobin through the RBC population. HbF does not participate in gel formation when mixed with HbS. This may explain in part the inhibitory effect of HbF on the sickling process in vivo.^{7, 12, 13}

A number of workers,^{5, 14-17} reported that short chain fatty acids (C3-C5) and their analogs are able to induce or stimulate the production of foetal haemoglobin in vivo.

Apart from their known biochemical functions, vitamins A, C, and E are known to be naturally occurring antioxidants that prevent peroxidation (auto-oxidation) of lipids exposed to oxygen.¹⁸⁻²⁰ Sickle-cell disease is known to be associated with membrane lipid abnormality. Long chain unsaturated fatty acids are known to increase membrane fluidity, decrease adhesion and aggregation of RBC.²¹

The role of antioxidant vitamins in preventing peroxidation of lipid especially in SCA subjects with normal levels of antioxidant vitamins and for the fact that presence of HPFH in SCA subjects has ameliorating effect prompted this study, to determine the serum levels of antioxidant vitamins A, C and E in SCA, HPFH-SCA and non-SCA children in Sokoto metropolis.

Patients and Methods

Sample collection

Blood samples were obtained randomly from confirmed sickle cell anaemic (HbSS) and foetal haemoglobin persistent sickle cell anaemic (HbSS+F) children within the age range of 9 months – 12 years, attending paediatric outpatient clinic, Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto.

Blood samples of apparently healthy and confirmed non-Sickle-cell anaemic (HbAA) children within the range of 9 months – 12 years attending Usmanu Danfodiyo University Staff Nurse/Primary School, Sokoto were obtained for analysis using systemic sampling technique. In all cases, the informed verbal and/or written parental consent and that of UDUTH and the school Headmistress were obtained before sample collection by the Paediatrician (NMJ). None of the children used in this study was on any form of preparation or medication containing vitamins A, C and E for at-least two weeks before the sample collection. The study was conducted between 1 August 2002 and 30 September 2002.

Preparation of sample

Three (3) millilitres of venous blood were collected from each subject into a stoppered vial containing EDTA. The samples were centrifuged at 3000 rpm for 5 minutes and the sera obtained. All sera were analyzed within one hour of collection. All chemicals used in this study were of high analytical grade.

Methods

Vitamin A was determined spectrophotometrically according to the method described by Neeld and Pearson²². The method uses trifluoroacetic acid that reacts with the conjugated double bonds of vitamin A to form a faint blue, short-lived compound that was measured spectrophotometrically at 620nm.

The method of Essien²³ was used for the determination of vitamin C. The method is based on the oxidation of ascorbic acid to dehydroascorbic acid that reacts with 2,4-dinitrophenylhydrazine to form a hydrazone, which when treated with sulphuric acid forms an orange-red colour, which is measured spectrophotometrically at 520nm.

Vitamin E was also determined according to the method adapted by Essien.²³ The method is based on the reduction of ferric ions to ferrous ions of ferric chloride, which forms red colour with, α, α -dipyridyl, which was measured spectrophotometrically at 460nm.

Differences in data were analyzed by the student t-test and Chi-square test. P value <0.05 was taken as significant.

Results

Thirty-eight children (male: female: 16:22) with SCA (HbSS), and 32 (male: female: 18:14) with non-SCA (HbAA) were studied. Of the 38 children with SCA, 11 (29%) had HbF, hence constituting the group, SCA + HPFH.

The age of non-SCA (HbAA) children ranged from 0.75 to 12 years with the mean (SD) of 7.7±(4.7) years while those of SCA (HbSS) and SCA + HPFH (HbSS+F) were between 0.75 to 12 years with

the mean (SD) of 5.9 ± (4.7) years and 1 to 12 years with mean (SD) of 6.9 ± (2.9) years respectively. Those children with HbSS+F are of older age group, when compared with the age groups of SCA children. This difference was significant (p<0.05).

The mean serum levels of vitamins A, C and E in foetal haemoglobin persistent sickle cell anaemic (HbSS+F), sickle-cell anaemic (HbSS) and non-sickle cell anaemic (HbAA) children is shown in Table 1. In all cases, the levels of the three-antioxidant vitamins were found to be insignificantly (p>0.05) higher in HbSS+F than in HbSS. In addition, the serum levels of the antioxidant vitamins in HbAA were found to be significantly higher (p<0.05) than those in HbSS and HbSS+F.

Table 1: Mean serum levels of vitamins A, C and E in HbSS+F, HbSS, and HbAA children

| Serum vitamin level(ug/dl) | Type of haemoglobin | | |
|----------------------------|---------------------|-------------|-------------|
| | HbSS+F (n=11) | HbSS (n=27) | HbAA (n=32) |
| Vitamin A | 27.5+8.9 | 26.4+4.6 | 45.3+6.2 |
| Vitamin C | 58.4+3.9 | 55.2+6.4 | 86.4+7.4 |
| Vitamin E | 16.2+4.2 | 15.3+3.4 | 84.2+4.2 |

Hb = haemoglobin

Discussion

The prevalence of HPFH of 29% in this study is consistent with what has been reported previously.¹⁰

¹¹ The presence of a significant amount of foetal haemoglobin within each red cell of an individual tends to impair sickling at physiologic oxygen tension¹³ and has also been noted to be clinically ameliorating for patients with sickle-cell anaemia.¹² Hence, majority of individuals with HbSS+F are known to be free from most of the severe clinical manifestations associated with sickle-cell anaemia.¹² It is therefore not surprising that in this study, children with HbSS+F were relatively older when compared to those with HbSS. One of the explanations may be that since they are less prone to severe clinical manifestations of SCA, tendency for them to survive into older age group becomes very high.

Much more low serum levels of antioxidant vitamins in HbSS compared to HbSS+F subjects makes the former group of children more prone to haemolytic crisis.¹² Also, the concentration of HbS in HbSS subjects is known to be higher than that in HbSS+F subjects indicating that there is more tendency of haemolysis in HbSS than HbSS+F.^{7,12,13}

The serum levels of the antioxidant vitamins in HbAA were found to be significantly higher than those in HbSS and HbSS + F subjects since the rate of haemolysis in the former group is much less. The slightly low level of the antioxidant vitamins observed in HbSS and HbSS+F children may be attributed to the utilization of the vitamins in preventing the peroxidation of the lipid,²⁴ necessary for decreasing the aggregation of the red blood cells or may be due to inadequate store or intake. Aggregation of the red

blood cells is a common feature in HbSS individuals and this leads to vaso-occlusive phenomenon more frequently than in the HbSS+F individuals.¹³

However, the roles of short- chain fatty acids supplementation of the diets of HbSS and HbSS+F children which have been known to induce or stimulate the production of HbF (foetal haemoglobin),^{5,14-17} shall be subject of our future communication.

In conclusion, the less depressed serum level of antioxidant vitamins A, C and E in HbSS + F children may be a contributory factor to their relatively stable clinical status. Therefore, it is recommended that HbSS + F and HbSS children should be placed on antioxidant vitamins A, C and E supplement in addition to routine folic acid and proguanil.

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