ORIGINAL ARTICLE

Red Cell Folate Status of Children with Sickle Cell Anaemia in Zaria, Nigeria

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ABSTRACT

Background:
Sickle Cell Anaemia (SCA) is the commonest and severest form of Sickle Cell Disease (SCD) affecting about 3% of the Nigerian population. Increased metabolic rate and protein turnover associated with SCA tends to deplete the body of micronutrients especially, body folate which is necessary for the synthesis of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). This necessitates folic acid supplementation to meet the body's requirements in SCA patients.

Aim and Objective:
To determine the red cell folate status of children with SCA and the effects of folic acid supplementation.

Materials and Methods:
This was a cross-sectional study carried out at the Paediatric clinic of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria – Nigeria over a six months period (June – December 2015). One hundred and seventy (170) SCA patients in steady state or in crisis state and 170 controls were recruited. A structured interviewer-administered questionnaire was administered. Socioeconomic status and folic acid intake and frequency of crisis were assessed and a general physical examination was carried out on each study participant. Blood samples were taken for RBC folate determination using electrochemiluminescence immunoassay (ECLIA); full blood count and differentials and blood films were also carried out using standard laboratory procedures.

Result:
Ninety-one percent of the SCA patients were taking daily 5mg of folic acid and 99.4% were regular on it. The mean RBC folate level of children with SCA (2005.3±1020.9 ng/ml) was significantly higher than that of the controls which was 838.3± 256.3 ng/ml (P = 0.001) and was also higher than the normal range (106-531ng/ml). There was no significant difference in RBC folate levels between SCA patients on folic acid and those who were not (P = 0.13). There was also no significant difference in the RBC folate level between SCA patients in steady or crises states (P = 0.73). The socioeconomic status of the SCA patients or the controls did not significantly affect their RBC folate levels (P = 0.1) and (P = 0.64) respectively. The mean haematocrit (HCT) was significantly lower in SCA (21.9%) in than the controls (31.7%), (P = 0.001). The RBC folate levels of the SCA did not significantly influence their haematocrit levels (P = 0.16).

Conclusion:
The red cell folate levels of patients with SCA were very high irrespective of their crisis state, folic acid intake, socioeconomic background, or haematocrit levels. The high red cell folate levels also found in the controls could suggest that folate intake from the dietary sources in the community might be adequate in this environment and routine folic acid supplementation may not be necessary. However, further studies, preferably multicentre, are needed before a policy on the routine use of folic acid supplementation in patients with SCA could be made.

Keywords: sickle cell anaemia, red blood cell, folate, folic acid

INTRODUCTION

Sickle Cell Anaemia is the commonest and severest form of SCD.[1] About 5% of the world's population carries the gene responsible for haemoglobinopathies.[2] Nigeria has the highest burden of the disease in the world with over 150,000 children born every year with SCD.[3] The homozygous state is found in about 3% of the population, [2,4] but a higher prevalence (11.87%) of homozygous state was reported in Kano metropolis and its suburbs in North-western Nigeria.[5] The mortality rate among SCA is about 5%, but could be as high as 38%, which is usually due to anaemia.[6,7] Sickle cell anaemia is characterised by chronic haemolysis and vaso-occlusion resulting in decreased live span of erythrocytes. Increased metabolic rate and protein turnover associated with SCA tends to deplete the body of micronutrients one of which is folate, which is involved in some methylation reactions including those in protein; deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) synthesis.[8] The prevalent low socioeconomic status, infections and infestation affect macro and micro nutrient availability which contribute to folate deficiency in the population. This necessitates folic acid supplementation to meet the body's increased requirement of SCA patients. Folic acid deficiency has been shown to complicate haemolytic anaemia [9], attributed to the demands of increased erythropoiesis and it is particularly liable to occur when dietary intake of folic acid is poor. Megaloblastic anaemia which responds to folic acid supplementation has also been demonstrated in SCA.[10,11] Therefore, routine folic acid supplementation for patients with SCA is widely recommended and became standard practice in many centres because of its low cost, apparent safety, and theoretical benefit.[12] Some studies have even recommended twice daily dosing of folic acid in cases of anaemic crises.[13] However, other studies have reported adequate folate stores in children with SCA not receiving folic acid supplements despite underlying haemolytic anaemia and concluded that routine folic acid prophylaxis is not necessary.[14] Although folic acid is widely administered to children with SCA, its optimal daily requirement has not been established.[15] Red cell folate levels, however, remain elevated for periods above forty days following discontinuation of supplements. [16,17] The measurement of folate in red blood cells is preferred since it reflects long-term folate status in the body compared to the plasma/serum folate, which may be influenced by the recent dietary intake.[18] This study aimed to determine red cell folate status of children with SCA in stable or crisis states.

MATERIALS AND METHODS

We conducted a cross-sectional study between June – December 2015 involving 170 children with SCA in steady (absence of infection, acute complicating factors or acute clinical symptoms or crisis for at least three months) [19] or in crisis state attending the haematology clinic in the Department of Paediatrics, ABUTH, Zaria. ABUTH is a tertiary and referral health centre in Kaduna State in North-western Nigeria. The Paediatric ward has a capacity of 90 beds. The Paediatric Haematology Clinic has over 1200 SCA patients. Approval of the Health Research Ethics Committee of ABUTH Zaria was obtained before the commencement of the study. Informed written consent of the parents or caregivers and assent were also obtained. Children who had a blood transfusion within three months preceding the recruitment, chronic diarrhoea (diarrhoea lasting more than 14 days), and children on medications known to affect folate metabolism e.g. cimetidine, carbamazepine, phenobarbitone, etc. were excluded from the studies. One hundred and seventy controls, who were children with the genotype AA who presented with acute illnesses that did not affect red cell folate were also recruited. The subjects were classified into different socio-economic classes based on Ogunlesi *et al* classification.[20] The nutritional status of the subjects was assessed based on their weight and height.[21-23]

Data collection: Relevant data were collected from all the children recruited using a structured interviewer-administered questionnaire. Demographic data, presenting complaints, history of folic acid supplementation and other medication and

nutrition histories were recorded. Physical examination was conducted on all the study participants which included anthropometry, vital signs and other physical signs were documented in the proforma. All the study participants had their non-fasting blood specimens collected using standard techniques.[24] Five millilitres of venous blood were collected by clean venepuncture from each patient via the antecubital vein using a plastic syringe into commercially prepared concentrations of ethylene di-amine tetracetic acid (EDTA) bottles. Each blood sample was mixed gently and thoroughly to prevent cell lysis and ensure anticoagulation. The blood samples were used to determine full blood count (FBC) and prepare haemolysate for red cell folate determination for all the study participants. In the controls, some of the blood was also used to determine their haemoglobin status. A FBC was analysed using - the Sysmex Xt 2000i an automated haematology analyser.[25] Haemoglobin electrophoresis of the controls was determined using cellulose acetate paper with a buffer at alkaline medium (pH of 8.0 - 9.0) as described by Adewuyi.[26] Red cell folate was assayed using Electrochemiluminescence immunoassay (ECLIA) analyser. The Elecsys folate III assay was performed following the directions given in the instrument manual.[27] The analyser automatically calculates the analyte concentration of each sample in ng/ml.

To calculate the folate concentration in the erythrocytes (RBC), the following equation was used.[27]

Red cell folate concentration =

$$\frac{folate\ concentration(analyser\ result)}{haematocrit} \times 100$$

Reference values = 106 – 531ng/ml (standard calibration provided by manufacturer).

Data analysis

Data on socioeconomic status and clinical information of the study participants were collected. The red cell folate levels, HCT, haematological parameters and nutritional status of the participants were also documented. The data were analysed using the statistical software EPI-info 3.5.3 version. Student's t-test was used to compare normally distributed continuous variables, e.g. differences in means and standard deviation, while differences in proportions were evaluated by Chi-square. The red cell folate levels of different groups of children were compared and a *P*-value of less than or equal to 0.05 was considered statistically significant.

RESULTS

One hundred and seventy children with SCA and an equal number of controls were enrolled. The patients with SCA consisted of 91 (53.5%) males and 79 (46.5%) females giving a male to female ratio of 1.15:1 while the control group had a male to female ratio of 1.24:1 (94 males and 76 females). The ages of both cases and control ranged from 6 months to 12 years. The mean age for SCA patients was 5.7± 3.3 years, while that of controls was 4.5 ± 2.8 years (*P* = 0.2).

Nine (5.3%) patients with SCA belonged to the upper socioeconomic class while 130 (76.5%) belonged to the lower socioeconomic class. Fourteen (8.3%) of the controls were in the upper socio-economic class while 122 (71.7%) belonged to the lower socio-economic class (*P* = 0.29).

One hundred and fifty five (91.2%) patients with SCA were on a daily 5mg folic acid supplement and only 1 (0.6%) of them was not taking the drug regularly. The mean duration of folic acid intake since diagnosis was 3.6± 3.1 years.

One hundred and thirty six (80%) of the patients were in a steady state, while thirty-four (20%) had crises {6 (3.5%) had anaemia and 28 (16.5%) had VOC} at presentation.

More SCA patients were underweight or severely underweight 31(18.2%) when compared to controls 24 (14.1%), but there was no significant difference between them (χ^2 = 1.06, *P* = 0.30). Although, more SCA patients were stunted or severely stunted 39 (22.9%) when compared to controls 36 (21.2%), there was no significant difference in the mean heights of the two groups (*P* = 0.69). The mean body mass index (BMI) -for-age was also not significantly different between patients and controls (*P* = 0.45).

The red cell folate levels for the patients with SCA ranged from 521 - 9673 ng/ml, while those of controls ranged from 545 - 2262 ng/ml. The mean red cell folate of patients with SCA (Table I) was 2005.3 ± 1020.9ng/ml, while that of the controls was 838.3 ± 256.3 ng/ml (*P* = 0.001).

Table I: Mean red cell folate levels of patients with sickle cell anaemia and controls

	SCA Mean ± SD	**Controls** Mean ± SD	***P*-value**
Red Cell folate (ng/ml)	2005.3±1020.9	838.3±256.3	0.001
Median	1995	786	
Mode	2780	752	

KEY: SCA= Sickle cell anaemia

One hundred and fifty-five (91.2%) patients with SCA, who were taking folic acid supplements, had mean red cell folate of 2042±1021.7 ng/ml, while 15 (8.8%) of the those not on folic acid had 1627 ±894 ng/ml (*P* = 0.13).

Table II shows the mean red cell folate levels of patients in the steady state and in crisis. There was no statistical difference between red cell folate level in VOC and anaemic crisis states (*P* = 0.73) and between the steady state and crisis (*P* = 0.08).

Table III shows that the majority of controls had red cell folate levels ranging from 501 ng/ml to 1000ng/ml, while half of the patients had red cell folate levels of more than 2000 ng/ml. There was a significant difference in the ranges of red cell folate levels of both patients and controls. (*P* = 0.0001). The mean red cell folate levels of patients with SCA were not significantly different from that of the controls in the various socioeconomic classes (F= 2.4, *P*= 0.1; and F =0.43, *P* = 0.64 respectively).

Table VII shows the relationship between red cell folate levels and the nutritional status of the SCA patients and the controls. There was no significant statistical relationship between the red cell folate levels and the weight-for-age Z-score (WAZ), height-for-age Z-score (HAZ) and BMI-for-age Z-score (BAZ) of the SCA patients or the controls. However, a significantly inverse correlation was observed between the ages of the controls and their red cell folate levels (r = –0.215 (*P* = 0.01). This indicates that the older a child with Hb AA is, the lower the red cell folate level.

Table II: Red cell folate levels of patients with sickle cell anaemia in steady and crises states

Diagnosis	**n = 91**	**Red Cell Folate ng/ml Mean (SD)**	**t-test**	***P*-value**
Anaemic crisis	6	1765 (1140.9)	0.3	0.73
VOC	28	1640.3 (713.2)		
Steady state	136	2091.1 (1046.8)		

VOC = vaso-occlusive crisis, SD= standard deviation, n=number of patients

Table III: Red cell folate levels of patients with sickle cell anaemia and controls

Red Cell Folate (ng/ml)	SS n (%)	AA n (%)	*P*- value
501 - 1000	29 (17.1)	143 (84.1)	0.0001
1001 - 1500	35 (20.6)	22 (12.9)	
1501 - 2000	21 (12.3)	2 (1.2)	
>2000	85 (50.0)	3 (1.8)	
Total	170 (100)	170 (100)	

χ^2 = 3, *P* = 0.0001, SS = Haemoglobin SS, AA = Haemoglobin AA

Table IV: Relationship between red cell folate of patients with sickle cell anaemia and controls and their nutritional status

	Red Cell Folate	
Nutritional status	**SCA r- value**	**Controls r-value**
WHZ	0.047	-0.043
HAZ	-0.055	0.057
BAZ	0.109	0.053
AGE	-0.123	- 0.215*

*Pearson correlation was significant at 0.01 level, SCA = Sickle Cell
WAZ = Weight-for-age Z- score, HAZ = Height-for-age Z- score, BAZ = BMI-for-age Z- score

There was a significant difference in red cell folate levels amongst children with SCA and controls with haematocrit levels between 15-30% (*P* = 0.0001). There was no significant correlation between red cell folate levels and haematocrit levels among patients with sickle cell anaemia (*P* = 0.16), but there was a significant correlation in red cell folate levels and haematocrit levels in controls (*P* = 0.001).

The mean haematocrit (Hct) level of the control group was significantly higher than that of the patients with SCA (*P* = 0.001). White blood cell (WBC) count, mean corpuscular haemoglobin concentration (MCHC), and platelet (Plt) counts of patients with SCA were also significantly higher than those of controls. There was however, no significant difference between the MCH and MCV of both patients and controls.

DISCUSSION

In this study, we document that the red cell folate levels of children with SCA were independent of folic acid intake, socioeconomic level, crisis state or nutritional status and that these children had a significantly higher red cell folate than controls with Hb AA (P=0.01). This study found generally high levels of red cell folate among the populations studied, but patients with SCA had higher levels than the controls. This could be attributable to the folic acid supplements that most patients with SCA take regularly. The high levels of red cell folate recorded in both patients and controls could also perhaps be associated with some common food items such as pasta, beverages, powdered milk, margarine and flour are now more widely fortified with folic acid as required by law in Nigeria.[27,28] Even though we did not measure the folate levels of these food

items. These food items are relatively cheap and so widely consumed in the society regardless of socioeconomic status.

This study found no significant difference in red cell folate levels between those taking folic acid supplements and those who were not. The high level of red cell folate found in SCA patients who were not on folic acid supplementation may be due to the improved dietary intake of folate from food fortification and fruits and vegetables in dietary intake.[28] This finding is similar to that of Kennedy *et al* [29] in the USA, who reported that there was no relationship between folate supplementation and red cell folate levels of patients with SCA, despite the much lower dose (1mg) of folic acid taken by his patients when compared with patients on 5mg folic acid, the dose taken by the patients in this study. Rodriguez-Cortes *et al* [14] in Dallas, USA, and Schnog *et al* [30] in Amsterdam also reported adequate red cell folate in SCA patients not taking a folic acid supplement and concluded that folate stores in children with SCA patients not receiving folic acid supplements were adequate despite the underlying haemolytic anaemia. Akinsulie [31] in Lagos, however, reported an insignificant reduction in the red cell folate amongst SCA patients who were not on folate supplementation, this could have resulted from malabsorption of folate from the diet of those affected children probably caused by enzyme defects or diseases of the digestive system, which are major causes of folate deficiency in humans [32] and not necessarily due to their SCA status.

Rabb *et al* [33] in Jamaica suggested that the policy of routine folic acid supplementation should be reviewed. Rodriguez-Cortes *et al* [14] reported that folate stores in the children with SCA who were not receiving folic acid supplements were adequate and similar to those in haematologically normal children. They found normal concentrations of red cell and serum folate and plasma homocysteine in these subjects. They also found no difference in folate levels in patients who experienced acute sickle cell complications, therefore concluded that tissue folate deficiency was infrequent and that folic acid supplements are not required in most children with SCA. Akinsulie *et al* [13] reported that the mean red cell folate of patients in painful crises was significantly higher than that of controls, but the mean red cell folate of some patients in anaemic crises was lower than that of controls. He suggested that the practice of daily 5mg folic acid supplementation should continue and should be given twice daily in the case of anaemic crises.

The controls recruited for this study were children who presented with acute illnesses, these illnesses could affect serum folate levels, but do not affect the folate levels in red blood cells as red cell folate reflects long-term folate status in the body.[18]

This study recorded no difference in the red cell folate levels in patients with SCA while in steady and their crises states or types of crises. This is similar to findings by Akinsulie [13] where those in VOC had normal or even significantly higher than normal red cell folate levels. Also, the patients in anaemic crisis in this study had a high mean red cell folate level inferring that the cause of symptomatic anaemia was not folate related, but it may likely be due to an acute cause that does not deplete the body's folate stores. This could also suggest that other causes of anaemia apart from SCA should be investigated and treated in patients with SCA presenting with symptomatic anaemia. This finding is however contrary to the finding of Akinsulie who reported low red cell folate levels in patients with SCA with anaemic crisis.[13] The low red cell folate levels could have been due to other causes e. g malabsorption of folic acid or other disease conditions, and not necessarily due to the SCA crisis.

There were significant differences in the ranges of red cell folate levels between patients with SCA and the controls. It was observed that half of the SCA patients had >2000ng/ml, while more than two-thirds of the controls had red cell folate levels between 501- 1000ng/ml. This difference could be due to the routine folic acid supplement in SCA patients. The lack of significant difference in red cell folate levels between the various socioeconomic classes of the two groups could again be due to the folic acid supplementation, folic acid is cheap, available, and thus affordable. Other studies [13,29,34,35] found no difference in

red cell folate levels between subjects from the middle and low-income classes. This study did not observe any significant relationship between the nutritional status of both patients and controls and their red cell folate levels. Liu and Augusta [36] reported similar findings, therefore, the poor nutritional status of the patients with SCA could be a function of repeated crises and not necessarily their food intake.

Folic acid supplements may also be responsible for the lack of a significant correlation in red cell folate that was observed in the different age groups of the patients with SCA. However, there was a statistically significant negative relationship among the ages of the controls and their red cell folate levels suggesting that the older the age the lower their red cell folate levels; this relationship is similar to that reported by Kennedy *et al*. [29]

This study recorded a mean haematocrit level among the patients with SCA that was significantly lower than that of controls similar to other studies in Lagos [31] and Benin.[19] Aneke *et al* found a mean haematocrit of 22.7%, while Sanjeev e*t al* and Sharon *et al* documented a haematocrit level of 23.1%.[37,38,39] Other studies also reported similar low haematocrit values in patients with SCA. [40,41] Chronic haemolysis, low erythropoietin response and shortened red cell survival may explain the low haematocrit observed in the patients with SCA in this study. Although our report recorded a significant difference in the mean red cell folate level between SCA patients and the controls; haematocrit levels between 15-30%, the same was not observed among patients with different levels of haematocrit. Akinsulie reported low folate level in patients with SCA that had anaemic crises, which might be due to other factors that deplete body folate especially malnutrition amongst these patients.[13] The normal MCV, MCH and MCHC observed in patients with SCA who were not on folic acid supplement suggests that folic acid deficiency is not the only cause of megaloblastic anaemia in these children.

CONCLUSION

The mean red cell folate level in patients with SCA was higher than that of controls. There was no significant difference in red cell folate levels between sickle cell anaemia patients who were on regular daily 5mg folic acid supplementation and those who were not. The red cell folate levels of the patients with SCA were independent of the steady state, crisis state or type of crisis, haematocrit levels and socioeconomic status of these patients. There was no significant relationship between the nutritional status of the patients with either red cell folate levels or age.

RECOMMENDATIONS

There is a need to establish a normal reference range for red cell folate levels in patients without sickle cell anaemia in Nigeria, using a standardised method, since values obtained are method dependent. Newly diagnosed patients with SCA should have their folate status determined at baseline before commencement of folic acid supplementation. Furthermore, they should have periodic assessment to determine the need for folic acid supplementation.

Limitations:

The number of patients with SCA who presented in crisis in this study was small despite the duration of the study, because many patients in crises did not fulfil the inclusion criteria.

Conflict of Interest:

None

Authors' Contributions:

YA conceived, designed and presented the idea. Data collection, analysis and interpretation and drafting of the manuscript was supported by JAF. EEE and RMA critically reviewed the concept note and the manuscript. All the authors approved the final version of the manuscript for publication.

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