ORIGINAL RESEARCH

Folate levels in children with sickle cell anaemia on folic acid supplementation in steady state and crises at a tertiary hospital in Enugu, Nigeria: a prospective, comparative study

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Abstract

Introduction

Folic acid supplementation is an integral aspect of the management of children with sickle cell anaemia (SCA) especially in Africa. In spite of this, there have been concerns about lower folate levels, especially during crisis.

Aim

To determine red cell folate levels of children with sickle cell anaemia in steady state and during crisis and compare with those with haemoglobin AA genotype.

Method

This study was prospective, hospital based, and comparative. Fifty children with sickle cell anaemia were recruited during crises and followed up until they met the criteria for attaining steady state. The controls were fifty children matched with those with SCA for age and gender and had haemoglobin AA genotype. Red cell folate estimation was done with the Electrochemiluminescence Immunoassay (ECLIA) method using the automated Roche Cobas e411 equipment.

Results

The median (IQR) red cell folate level in children during sickle cell crisis was 265.95 (134.50) ng/ml, which was significantly lower than the median (IQR) of 376.30 (206.85) ng/ml obtained during steady state. Most children with SCA (41 out of 50) had significantly higher folate levels during steady state (T=1081, Z-score= -4.660, $p < 0.001$). Median level of red cell folate was lower during anaemic crisis compared to vaso-occlusive crisis, though not significantly so (N(50), U = 214.00, Z-score= -1.077, $p = 0.305$). The median red cell folate level of normal controls was 343.55 (92.90) ng/ml, which was significantly lower than the 376.30 (206.85) ng/ml obtained during steady state (N(50), U= 209.00, Z-score= -7.177, $p < 0.001$).

Conclusion

Median red cell folate levels of the study participants were within normal limits, though most children with SCA had significantly higher levels during steady state compared to crisis. Normal controls had significantly lower red cell folate levels than the children with SCA during steady state.

Keywords: Red cell folate, sickle cell anaemia, steady state, crises.

Introduction

Sickle cell anaemia (SCA) is the commonest genetic disease worldwide[1]. About 3 in 100 newborn babies in Nigeria are born with sickle cell anaemia, with a total of over 150,000 babies per annum[2]. Three percent of the Nigerian population (over 6 million people) have sickle cell anaemia.1 Many children with sickle cell anaemia are in relative good health most of the time. This is otherwise known as the steady state[3]. This is interrupted occasionally by crises, especially the vaso occlusive type, which is the hallmark of SCA[3]. Crises are defined as episodes of acute illnesses characterized by worsening of clinical features of sickle cell anaemia, such as pain, anaemia, or jaundice[4].

Folate is a water-soluble B-vitamin necessary for activation of single carbon compounds for the production of purines which are required for synthesis of RNA and DNA[5]. It is also necessary for production of thymidylate which is needed for synthesis of DNA, and conversion of homocysteine to methionine[5]. It is a key requirement for proper cell division and production of erythrocytes. The average half-life of erythrocytes is reduced from 120 days in unaffected individuals to 15 to 20 days in SCA[6]. The half-life is further reduced during sickle cell crises, leading to increased activity of the bone marrow in order to replace the destroyed red blood cells[4]. This results in depletion of folate, and a greater likelihood of folate deficiency[7]. As a result of this, children and adults with sickle cell anaemia receive one to 5 mg of folic acid daily on the premise that this dose will be sufficient to restore the depleted folate levels and aid in production of erythrocytes[8]. In spite of the folic acid supplementation, folate deficiency has been documented in Nigerian children with SCA[9-11]. This is probably due to low folate content of

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the traditional Nigerian diet due to overcooking of vegetables and absence of national policies on food fortification with folic acid[12].

Studies done so far have reached no consensus as to whether there is folate deficiency in SCA, or if the folate level is lower during crisis[8-9,13-14] This is important because folate deficiency leads to hyperhomocystinaemia, which increases the frequency of thrombotic events such as vaso-occlusive crises and adversely affects the outcome of the disease[15]. The findings from this study will throw more light on whether there is need or otherwise to discontinue, continue, or increase the dose of folic acid in children with SCA, especially during crisis.

The primary aim of the study was to compare the red cell folate levels in children with SCA in steady state and during crisis and also to compare the folate levels in children with SCA during steady state with that of children with haemoglobin AA genotype to detect any significant differences.

Methods

The study was prospective and comparative, conducted at the University of Nigeria Teaching Hospital (UNTH), Enugu over a seven-month period extending from September 2018 to March 2019.

Determination of sample size

The sample size was determined using the formula for comparing two means[14]

$$n = 1 + \frac{1}{K}\left(\sigma \frac{Z_{1-\alpha/2}+Z_{1-\beta}}{\mu A-\mu B}\right)^2$$

This yielded a sample size of 50 in each group.

Study population/inclusion criteria

Fifty children with sickle cell anaemia aged two to seventeen years, were recruited during crises and had their erythrocyte folate estimated. They were followed up until they fulfilled the criteria for attaining steady state, (defined as absence of any painful episode requiring treatment in the hospital in the preceding one month, absence of fever or any inter-current illness at presentation or in the preceding one month, absence of blood transfusion in the preceding four months, not receiving any antibiotics or other medications that affect blood indices at time of recruitment)18 when their erythrocyte folate estimation was again estimated. These children with SCA were receiving 5mg daily folic acid supplements even during hospital admission. Fifty children who were apparently healthy and were matched with the children with SCA for age and gender, with haemoglobin AA genotype following haemoglobin electrophoresis done at recruitment served as controls. The controls were children who were on follow up at the Children Outpatient clinic, having recovered from acute illnesses like upper respiratory tract infections and malaria.

Exclusion criteria

Children who were receiving medications which affect folate levels such as antacids, H2 blockers, carbamazepine, phenytoin, and methotrexate, children who were transfused less than three months before recruitment, and children who had diarrhoea that lasted for more than fourteen days in the preceding one month were excluded from the study.

Ethical considerations

Ethical approval was obtained from the Health Research Ethics Committee of UNTH (NHREC/05/01/2008B/FWA00002458-1RB00002323, issued on 28/2/2018). Informed written consent and assent were obtained from care-givers and study participants as appropriate.

Definition of study subgroups

The children with SCA were classified into either the anaemic crisis or vaso-occlusive crisis category based on their major presenting complaints. The diagnosis made using a combination of clinical examination and laboratory investigations. Children with SCA who presented with bone or abdominal pain, irrespective of the severity, were grouped into the vaso-occlusive crisis category, while those who presented with worsening jaundice, passage of cola-coloured urine (haematuria on Mission expert COMBI-12 urinalysis strip), severe pallor (packed cell volume <15% or haemoglobin <5g/dl), increasing liver and spleen size, or features of anaemic heart failure (tachypnoea, tachycardia, tender hepatomegaly, displaced apex beat) with or without bone or abdominal pain were grouped into the anaemic crisis category. Criteria for steady state were: absence of any painful episode requiring treatment in the hospital in the preceding one month, absence of fever or any inter-current illness at presentation or in the preceding one month, absence of blood transfusion in the preceding four months, not receiving any antibiotics or other medications that affect blood indices at time of recruitment.18

Laboratory equipment

Whole blood samples (non-fasting) were taken using standard laboratory precautions for red cell folate estimation with the Electrochemiluminescence Immunoassay (ECLIA) method using the automated Roche Cobas e411 equipment. Red cell folate levels less than 140 ng/ml were regarded as low, while levels between 140 and 620 ng/ml were categorized as normal[19].

Method of statistical analysis

Analysis of data was done using the Statistical Package for Social Sciences version 20 (SPSS 20 Chicago). Categorical data were summarized as frequencies and percentages. Chi square and Fisher's test were used to test association between categorical variables. Kolmogorov-Sminorv test done on red cell folate levels to ascertain normality revealed that they were not normally distributed. Red cell folate levels in the different subgroups were summarized as median and inter-quartile range. Wilcoxon signed rank test was used to compare median levels of red cell folate in the same children with SCA during steady state and crisis. Mann Whitney U test was used to compare median levels in steady state and in normal controls, and median folate levels in the different types of crisis. Significant level was regarded as $p < 0.05$.

Results

Fifty children with SCA and their age and gender matched controls, comprising 58% males and 42% females, (M: F-1.38:1) were studied. The ages of children with and without SCA ranged from 2 years to 17 years, with a median of 10.00 (5.25) years. A greater proportion (48%) of the study population were aged between 7 and 11 years.

Table 1: Socio-demographic characteristics of study participants

Socio-demographic characteristic	N (%)		Test statistic	p-value
	Normal controls N=50	Children with SCA N=50		
Gender				
Male	29(58.0)	29(58.0)	0.000 [a]	1.000
Female	21(42.0)	21(42.0)		
Total	50(100.0)	50(100.0)		
Age (years)				
2 to 6	6(12.0)	6(12.0)	0.125[b]	1.000
7 to 11	24(48.0)	24(48.0)		
12 to 16	16(32.0)	16(32.0)		
>16	4(8.0)	4(8.0)		
Total	50(100.0)	50(100.0)		

[a]: Fisher's exact level of significance, [b]: chi square.

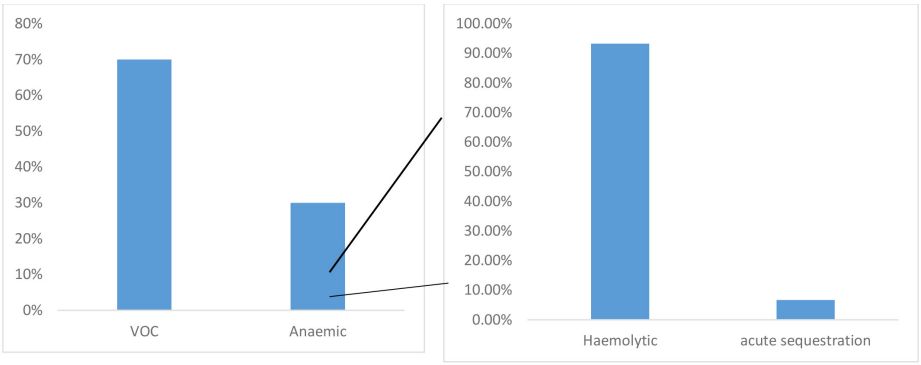

Figure 1: Classification of subjects based on type of crisis

Table 2: Comparison of folate levels in participants with sickle cell crisis and steady state using Wilcoxon signed rank test

Study group	Ranks	N=50	Median (ng/ml)	Inter-quartile range (ng/ml)	Z-score	p-value
Steady state-crisis	Negative	8	376.30	206.85	-4.660	<0.001*
	Positive	41	265.95	134.50		
	Ties	1				
	Total	50				

*Z-value: , * significant*

Table 3: Comparison of folate levels in children with SCA during steady state with normal controls

State	N = 100	Median (ng/ml)	IQR (ng/ml)	Mann –Whitney U	Z-score	p-value
Steady state	50	376.30	206.85	209.00	-7.177	<0.001*
Normal controls	50	343.55	92.90			
Total	100					

** Significant, IQR: Inter-quartile range*

Classification of subjects based on type of crisis

Vaso-occlusive crisis was the most common type of crisis observed, seen in 70% of the children, while 30% had anaemic crisis. Amongst the subjects with anaemic crisis, 6.7% had acute sequestration crisis, while 93.3% had haemolytic crisis as seen in Figure 1. None of the subjects had aplastic crisis.

Folate levels of the study population.

The median (IQR) red cell folate level during steady state was significantly higher than the level in crisis (T=1081, Z-score= -4.660, p < 0.001). Most (41) of the children with SCA had higher red cell folate levels in steady state than during crisis as seen in table 2. The median red cell folate in normal controls was significantly lower than the value obtained in the during steady state (U= 209.00, Z-score= -7.177, p <0.001) as seen in table 3. The median red cell folate level in anaemic crisis was lower than the level in vaso-occlusive crisis, though this was not significant (U = 214.00, Z-score= -1.077, p = 0.305) as seen in table 4.

Folate status of the children with SCA

Folate levels were below normal limits in 8% of the children during crisis, but normalized when they were followed up to steady state. None of the normal controls had folate deficiency. The difference in proportion of children in steady state and crisis with folate deficiency was not significant (Fisher's exact level of significance= 4.167, p = 0.117) as shown in table 5. Of the fifteen children having anaemic crisis, three (20%) had folate deficiency, while one (2.9%) out of the thirty-five children with VOC had folate deficiency. The odds of being folate deficient was 8.5 times higher during anaemic crisis than in VOC. This was however, not significant (95% C.I 0.805 - 89.750, p = 0.075).

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Table 4: Comparison of folate levels in children with vaso-occlusive and anaemic crises

Type of crisis	N=50	Median (ng/ml)	IQR (ng/ml)	Mann – Whitney U	Z-score	p-value
Vaso-occlusive	35	272.90	143.90	214.00	-1.027	0.305
Haemoytic	15	242.20	131.60			
Total	50					

IQR: Inter-quartile range

Table 5: Folate status of children with SCA

Children with SCA	Folate status N(%)		Total N(%) N=100	Fisher's	df	OR	95% C.I	p-value
	Low N=4	Normal N=96						
Crisis state	4 (8.0)	46 (92.0)	50 (100.0)	4.167	1	-	-	0.117
Steady state	0 (0.0)	50 (100.0)	50 (100.0)					
Type of crisis	N=4	N=46	N=50					
Anaemic	3 (20.0)	12 (80.0)	15 (100.0)	4.193	1	8.500	0.805 - 89.750	0.075

Discussion

This prospective, comparative study compared red cell folate levels in children with SCA during crisis and steady state with that of children with haemoglobin AA genotype to detect any significant difference in the folate levels. The children with SCA were receiving folic acid supplements at a daily dose of 5 mg even during hospital admission.

During crisis state of sickle cell anaemia, the median level of RBC folate was within normal range. This was similar to the observations of Akinsulie[9] and Olaniyi et al[17] in Lagos and Ibadan respectively. Though sickle cell crisis is characterized by haemolysis which could increase the demand for folate to replace the destroyed cells, the normal mean red cell folate levels could be due to the folic acid supplements which the subjects were receiving.

The median level of RBC folate in children during steady state was within standard limits, and in keeping with findings of Akinsulie in Lagos[9]. This was lower than the value observed by Abubakar in Zaria[20]. In addition, most of the children with SCA had significantly higher folate levels during steady state compared to crisis. This has been corroborated by earlier studies[9,17,20]. These findings were probably due to the absence of severe haemolysis and repeated infections which could lower folate levels in steady state when compared to crisis. Repeated infections could lead to severe haemolysis, as the macrophages engulf infected erythrocytes and reduce the amount of erythrocytes in circulation[21]. Following severe haemolysis, there is an attendant increase in activity of the bone marrow to replace the destroyed cells[7]. This could deplete the folate stores, and increase the likelihood of folate deficiency[7]. The finding of folate within standard limits during steady state could suggest that the folic acid supplementation dose is enough to maintain the tissue stores of folate.

The median level of red cell folate in normal controls was significantly lower compared with that in children with SCA in steady state. This compares favourably with the findings of Akinsulie[9] and Abubakar[20] in Lagos and Zaria respectively. The relatively lower folate levels compared to their steady state SCA counterparts may be due to destruction of folate in their food by excessive boiling[12], in addition, the normal controls were not receiving folic acid supplements. This implies that folic acid supplementation ensures availability of folate in children with SCA in view of the fact that they have shortened red cell half-life which increases red cell turn-over with increased folate demand since the body does not store folate in significant amounts.

In anaemic crisis, the RBC folate levels were lower than the values obtained during vaso occlusive crisis. The difference was probably not significant due to the small sample size in the group with anaemic crisis. This was supported by findings of Akinsulie[9] in Lagos. Plausible explanation for the lower folate levels during anaemic crisis could be due to the rapid reduction in the number of erythrocytes during haemolysis, and the increased demand of folate for erythropoiesis, which depletes the folate stores and increases the risk of folate deficiency[7].

Eight percent of the children with SCA had folate deficiency during sickle cell crisis. The prevalence of folate deficiency during crisis compares favourably with findings documented by Liu[23] and Watson-Williams[10], but varies considerably with the report of Kennedy et al[24] who observed a higher prevalence rate of 15% in children with SCA. The odds of being folate deficient was 8.5 times higher in anaemic crisis compared to VOC. This was however not significant, probably due to the small sample size studied. Akinsulie[9] in Lagos also found a higher prevalence of folate deficiency in subjects with anaemic crisis. The finding of more subjects with folate deficiency during anaemic crisis could

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be explained by the severe haemolysis which characterizes anaemic crisis. This leads to increased demand of folate for DNA synthesis and production of new erythrocytes to replace the destroyed ones[7]. Depletion of the folate stores could increase the risk of folate deficiency. The higher prevalence of folate deficiency observed during anaemic crisis despite folate supplementation suggests that there may be need for increased dose of folate during anaemic crisis, in order to replenish tissue stores.

Folate deficiency was neither observed in normal controls nor in children with SCA in steady state. This was similar to the findings observed by Liu[25] who found no folate deficiency in the normal controls. Interestingly, other studies reported high prevalence of folate deficiency during steady state and in normal controls[9,11]. The lower prevalence of folate deficiency obtained during steady state could be due to the stable state which the body is in during steady state sickle cell anaemia, despite the reduced half-life of the erythrocytes to 15 to 20 days.7 As a result of this, the rate of destruction of erythrocytes and erythropoiesis does not overwhelm the tissue stores of folate, coupled with the folic acid supplementation they received. The absence of folate deficiency in the normal controls could be explained by the fact that these children had haemoglobin phenotype AA, which has a half-life of 120 days, and the rate of destruction of the effete erythrocytes in children with AA phenotype is not severe enough to deplete the folate stores and cause deficiency of folate[7].

This study is not without limitations. The small sample size in the subgroup with anaemic crisis could have limited statistical deductions. The exact number of children with SCA who had both vaso-occlusive and anaemic crises could not be separated from those who had only anaemic crisis and this could account for the lower folate levels obtained during anaemic crisis. Also, study participants were not matched for social class and this could have affected the results since social class could affect dietary choices. These concerns should be addressed in further studies on the subject.

In conclusion, most of the children with SCA had higher folate levels in steady state, compared to crisis. Normal controls had significantly lower red cell folate levels than the children with SCA during steady state. More studies with larger sample size are needed for recommendations on folic acid supplementation.

Declarations

Ethical approval and consent to participate

Ethical consent was obtained from the Health Research and Ethics Committee of University of Nigeria Teaching Hospital, Enugu. Written informed consent was obtained from the parents and care-givers of the study participants. Assent was obtained from the study participants aged 7 years and above.

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Declaration of conflicting interests

The authors declare that there is no conflict of interest.

Availability of research materials

The datasets associated with this research are available on reasonable request from the corresponding author.

Author's contributions

U.N and C.N were involved in the conceptualization of the work, collection, analysis, and interpretation of data, drafting of the final copy for publication.

V.O, B.F, A.I, and I.E were involved in the conceptualization of the work, collection and interpretation of data, they also made corrections to the draft and approved the final copy for publication.

All authors agree to be responsible for the authenticity of the work and are available to answer any questions regarding the work.

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