Original Research Article

Relationship between folate status and complete blood count (CBC) parameters in sickle cell anaemia (SCA) at steady state in Aminu Kano Teaching Hospital, Kano, Nigeria

ABSTRACT

Aim: To determine the relationship between folate status and CBC parameters in SCA patients at steady state

Design: Comparative cross-sectional study

Setting: Departments of Haematology and Blood Transfusion and Paediatrics, Aminu Kano Teaching Hospital, Kano, Nigeria between April and August, 2017.

Methodology: One hundred and ten (110) each of SCA patients (subjects) in steady state and their age and sex matched controls were enrolled. Haemogram and folate levels were determined. Data were analysed with SPSS version 21.0 and p≤0.05 was considered significant.

Results: The mean age of the participants was 15.2±7.4 years with a range of 4 – 32years. There was no significant difference between the SCA patients and controls on basis of age, sex and level of education (p >0.05). The prevalence of folate deficiency among SCA patients was 46.4% (serum) and 49.1% (RBC) compared to controls 22.0% (serum) and 22.9% (RBC), p<0.05. SCA patients were more likely to develop folate deficiency than controls [OR (95% CI) for serum 3.1(1.7 to 5.5) and RBC 3.2(1.8 to 5.8)], p<0.05. Red cell folate deficiency is associated with low haematocrit and high RDW, while serum folate deficiency was associated with low haematocrit, p<0.05.

Comment [DGR1]: This is a cross-sectional study; we can only talk of association

Conclusion: Despite routine prescription of folic acid, SCA patients had a higher prevalence of folate deficiency and this was associated with lower haematocrit. We therefore recommend that, physicians should device criteria for assessing compliance with routine prescription and do folate assay for patients with persistent high RDW and low haematocrit in steady state.

Comment [DGR2]: Compliance/ adherence was not studied; so it is not appropriate to conclude from assumptions; authors would rather conclude by assessing need for adherence assessment and other factors such as dietary intake and recurrent infections in patients with high RDW

Keywords: sickle cell anaemia, folate status, complete blood count, steady state, kano, nigeria

1. INTRODUCTION

Individuals with conditions associated with excessive cell turnover such as sickle cell anaemia (SCA) are at risk for folate deficiency because of the role folate plays during normal cellular proliferation like haemopoiesis.[1-3] Increased erythropoietic activity meant to compensate for shortened red cell survival in SCA patients increases folate requirement with attendant consequences of developing folate deficiency.[3-5] Anaemia, macrocytosis and/or pancytopaenia are some of the peripheral blood abnormalities reported in folate deficiency and these can be detected on complete blood count (CBC).[3,6-7] This vicious cycle of folate deficiency and abnormal blood count parameters is particularly important for SCA patients in our environment where factors such as poor dietary intake, infections and repeated pregnancy can adversely affect the relation between folate and blood parameters.[4,8-10] Detailed understanding of this relationship will help to identify patients in whom folate assay may be indicated from the result of readily available and more affordable CBC test. This approach will save cost and prevent complications of folate deficiency. There is no study in this environment that previously determined the relation between folate status and CBC parameters among SCA patients in steady state, hence the need of the current study.

2. MATERIAL AND METHODS

This was a comparative cross-sectional study conducted among 110 each of SCA patients in their steady state as well as age and sex matched controls with AA haemoglobin. Patients with SCA were enrolled at adults and paediatrics haematology clinics of Nigerian Teaching Hospital in Kano, while controls were recruited from donor and well paediatric outpatient clinics of the same hospital from April to August, 2017.

Exclusion criteria for sickle cell patients were non-SS haemoglobin phenotype, pregnancy, use of hydroxyurea and anticonvulsant drugs. Also, excluded were SCA with hypertension, liver and renal diseases as well as those with HIV, Hepatitis B and C infections. All prospective blood donors who were deferred from donation and children with febrile illness or those tested positive to HBV, HCV and HIV were excluded from the study.

Clinical data covering evidence of hypertension, liver and renal diseases as well as drug history were obtained from the hospital case notes of all the SCA patients. Five milliliters of venous blood was collected from each participant and 2.5mls each was dispensed into plain and K2-ethylenediaminetetraacetic acid (EDTA) bottles. All samples were processed within 4hours of collection. Complete blood count was conducted with Swelab Alfa 3-part differentials Coulter (Boule Medical Diagnostics, Sweden) and reticulocyte count was determined manually. Serum harvested from clotted samples were stored at -20^{0}C for serum folate assay while haemolysate was prepared from EDTA samples by adding 0.1ml of blood into 2ml of 0.2g/dl ascorbic acid solution to obtain 1: 20 dilution.[11, 12] This was gently mixed and kept at room temperature in the dark for 60 to 90 minutes and then frozen at -20^{0}C for red cell folate assay.[11, 12] Folate concentration was determined through electro-chemi-luminescence technology on Elecsys 2010 (Roche Diagnostics, USA). Analytical control specimens (Universal pericontrol I and II) were included with each batch of test for folate assay. Human immunodeficiency virus, Hepatitis B and C were screened with Determine, Ascon and Healgen respectively.

In this study steady state is defined as absence of febrile illness, sickle cell related crises in the preceding 6 weeks and blood transfusion in the last 3 months. Folate deficiency is defined as red cell folate less than 100ng/ml and/or serum folate less than 6ng/ml according to WHO guidelines. [13]

Informed written consent was obtained from adult participants and parental/guardian consent and child assent were obtained from paediatric participants. Ethical approval was obtained from Ethical Review Board of the Hospital.

Data analysis was performed using Statistical Package for Social Sciences (SPSS) version 21.0 (IBM Corp. Armonk, NY) and results presented as mean ±SD, proportion and percentages. Independent sample t-test was used to compare means, χ^2 was used to test for association and logistic regression analysis was conducted to estimate risk. A confidence interval of 95% was used and $p < 0.05$ was considered significant.

3. RESULTS

The mean age of the participants was 15.2 ± 7.4 years with a range of 4 – 32years.There is no statistically significant difference between SCA patients and controls in any of the tested socio-demographic variables ($p > 0.05$). [Table 1]

The overall prevalence of folate deficiency among the study participants was 36.1% for red cell folate and 34.2% for serum folate. The SCA patients had a prevalence of 49.1% and 46.4% compared to controls with 22.9% and 22.0% for red cell and serum folate respectively. Patients with SCA were at least three times more likely to have folate deficiency than controls [OR (95% CI) for serum 3.1(1.7 to 5.5) and RBC 3.2(1.8 to 5.8)], p<0.05 [Table 2].

The relation between folate status and CBC parameters in SCA patients is presented in [Table 3]. Among SCA patients, those with folate deficiency had lower haematocrit and higher red cell

distribution width (RDW) compared to those with normal folate levels ($p < 0.05$). Also, red cell folate deficiency is associated with low haematocrit and high RDW, whereas serum folate deficiency was associated with low haematocrit ($p < 0.05$).

Table 1: Socio-demographic characteristics of the population

Variables		Sickle cell group		Control group		*P* - value
		Number (N)	Percentage (%)	Number (N)	Percentage (%)	
Sex	Male	58	52.7	51	46.4	0.07
	Female	52	47.3	59	53.6	
	Total	110	100.0	110	100.0	
Tribe	Hausa/Fulani	108	98.2	110	100.0	0.21
	Yoruba	2	1.8	0	0.0	
	Igbo	0	0.0	0	0.0	
	Others	0	0.0	0	0.0	
	Total	110	100.0	110	100.0	
Educational level	Primary	40	36.4	24	22.2	0.32
	Secondary	51	46.4	65	60.2	
	Tertiary	18	16.4	19	17.6	
	Non formal	1	0.9	0	0.0	
	Total	110	100.0	110	100.0	
Participants occupation	Student	96	87.3	95	88.0	0.14
	Petty trader	8	7.3	9	8.3	
	House wife	2	1.8	1	0.9	
	Civil servant	2	1.8	3	2.8	
	Artisan	2	1.8	0	0.0	
	Total	110	100.0	108	100.0	
Estimated monthly income (N)	≤12,000	4	4.1	5	4.5	0.08
	>12,000	94	95.9	105	95.5	
	Total	98	100.0	110	100.0	
Fathers occupation	Petty trader	16	14.5	21	19.1	0.06
	Business	40	36.4	33	30.0	
	Civil servant	22	20.0	20	18.2	
	Artisan	32	29.1	36	32.7	
	Total	110	100.0	110	100.0	
Mothers level of education	Primary	12	27.3	10	22.7	0.45
	Secondary	2	4.5	3	6.8	
	Tertiary	14	31.8	16	36.4	
	Non formal	16	36.4	15	34.1	
	Total	44	100.0	44	100.0	

Table 2: Prevalence of folate deficiency among study participants

Variables		Deficient Number (%)	Normal Number (%)	χ^2	*P* - value	OR (95% CI)
Red cell folate	Sickle cell	54 (49.1)	56 (50.9)	16.24	0.00	3.2 (1.8 – 5.8)
	Control	25 (22.9)	84 (77.1)			
	Total	79 (36.1)	140 (63.9)			
Serum folate	Sickle cell	51 (46.4)	59 (53.6)	14.41	0.00	3.1 (1.7 – 5.5)
	Control	24 (22.0)	85 (78.0)			
	Total	75 (34.2)	144 (65.8)			

Statistically significant $p < 0.05$

χ^2 = Chi- square, OR = Odd ratio, CI = Confidence interval

Table 3: Relation between folate status and complete blood count of SCA patients in steady state

Parameters	Red cell folate (Mean ± SD)		*P* - value	Serum folate (Mean ± SD)		*P* - value
	Deficient (N = 54)	Normal (N = 56)		Deficient (N = 51)	Normal (N = 59)	
Haematocrit (%)	24.1 ± 4.7	26.6 ± 6.9	0.03	23.8 ± 4.7	26.7 ± 6.8	0.01
WBC x 10^9/L	13.3 ± 5.2	12.7 ± 4.2	0.57	13. 4 ± 5.1	12.7 ± 4.4	0.47
Neutrophil x 10^9/L	6.6 ± 2.8	6.3 ± 2.4	0.49	6.8 ± 2.7	6.3 ± 2.4	0.27
Lymphocyte x 10^9/L	6.0 ± 2.6	5.3 ± 2.3	0.16	6.0 ± 2.6	5.4 ± 2.5	0.22
Platelet x 10^9/L	357.6 ± 167.3	322.3 ± 144.1	0.24	345.4 ± 173.1	334.6 ± 141.3	0.72
MCV (fL)	81.8 ± 8.5	83.8 ± 8.4	0.21	82.2 ± 8.7	83.4 ± 8.3	0.46
MCH (g	28.5 ± 3.5	29.3 ± 3.5	0.23	28.8 ± 3.5	29.1 ± 3.6	0.66
MCHC	34.8 ± 1.3	34.9 ± 1.8	0.69	35.0 ± 1.2	34.8 ± 1.9	0.60
RDW (%)	24.4 ± 4.4	22.8 ± 3.9	0.04	24.4 ± 4.5	22.9 ± 3.9	0.07
Reticulocyte count (%)	4.1 ± 2.9	4.0 ± 2.7	0.83	4.4 ± 3.0	3.8 ± 2.6	0.33

Statistically significant $p < 0.05$

MCH = mean cell haemoglobin, MCHC = mean cell haemoglobin concentration, MCV = mean cell volume, RDW = red cell distribution width, WBC = white blood cell

4. DISCUSSION

This study reported a high prevalence and increased risk of folate deficiency among SCA patients at steady state and importantly, association between folate deficiency and low haematocrit and high RDW.

The finding of higher prevalence of folate deficiency among SCA patients compared to normal controls from similar socioeconomic background was previously reported in studies from resource affluent nations. [4, 9, 14] This notwithstanding the prevalence of folate deficiency in those studies was between 13 to 15% which is lower than the prevalence reported in our study. The higher prevalence of folate deficiency among participants in our study is expected as the accessibility and utilization of good health care services are far better in resource affluent nations compared to our environment.[6, 10] This high prevalence of folate deficiency among SCA patients assessing care at our center is in spite of the routine folic acid prescription which is the standard of care, to all SCA patients and adequate quantity of biologically active folate in folic acid supplements.[9, 13] The clinical implications of folate deficiency in SCA patients include severe anaemia, recurrent transfusion and growth retardation in children, increased risk of stroke and central nervous malformations in foetuses of pregnant SCA patients with folate deficiency.[3, 9, 13] Moreover, folate deficiency may be a risk factor for the occurrence, persistence and delayed healing of sickle cell leg ulcers since adequate folate is necessary for DNA synthesis, cellular proliferation and epithelialization in wound healing processes.[15]

Findings of a statistically significant lower haematocrit and higher RDW among folate deficient SCA patients in this study could be sequel to megaloblastic anaemia arising from folate deficiency leading to dimorphic in red cell population made of macrocytic and normocytic cells.[6] The absence of any significant difference in other CBC parameters between SCA patients with deficient and normal folate status could not be readily explained, since folate

deficiency frequently presents with high mean cell volume, leucopaenia, thrombocytopaenia and reticulocytopaenia among other abnormalities in peripheral blood.[2, 4, 5, 9, 14] The findings in this study were in keeping with that of Liu et al, who reported lower haematocrit and reticulocyte count among folate deficient SCA patients with no significant difference in other parameters.[4] It is important for clinicians to note that, with the exception of high RDW most of these abnormalities occur late in folate deficiency and therefore should not be awaited before evaluating SCA patients with suspected folate deficiency.[6] This will serve as pre-emptive strategy of preventing the complications of folate deficiency among SCA patients.

The contribution of other co-morbidities that could affect folate level such as malabsorption syndrome and vitamin B12 deficiency were not screened for and these are some of the limitations of the study.

5. CONCLUSION

The prevalence of folate deficiency among SCA patients of whom folic acid is routinely prescribed is high and this is associated with abnormal findings on CBC test. We therefore recommend that, physicians should device objective criteria for assessing compliance to routine prescription and possibly by folate assay for SCA patients with persistently low haematocrit and high RDW.

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COMPETING INTEREST: Nil

CONSENT: Informed written consent was obtained from adult participants and parental/guardian and child assent were obtained from paediatric participants.

ETHICAL APPROVAL: Was obtained from Ethical Review Board of the Aminu Kano Teaching Hospital, Kano, Nigeria.

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