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Effect of Bone Pain Vaso-occlusive Crises on Red Blood Cell Parameters of Sickle Cell Anaemia Patients

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Abstract

Background: Sickle Cell Anaemia (SCA) is an inherited haemoglobin disorder characterized by shortened red cell life span and altered red blood cell parameters due to the red cell sickling, vaso-occlusion and chronic haemolysis occasioned by the disease. The occurrence of bone pain vaso-occlusive crises may further alter these parameters; an occurrence that highlights on the pathophysiology of SCA and worth noting in the management of affected patients. Aims and Objectives: This study was performed to determine the effect of bone pain vaso-occlusive crises on red blood cell parameters of SCA patients. Study Design: A longitudinal study. Settings: Fifty SCA patients receiving care at a tertiary hospital in Sokoto, Nigeria were consecutively enrolled while in their steady clinical state and followed up till when they re-presented during the bone pain vaso-occlusive crises state. Materials and Methods: Structured proforma was used for clinical data capturing while red blood cell parameters (red blood cell count (RBC), haemoglobin (Hb) concentration, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW) were determined via automation using Nortek 3-part haematology analyser. Leishmann stained peripheral blood smears were examined to cross-check morphological details of the red blood cells. Statistics: Data analysis was performed using Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM Corp, Armonk, NY, USA) and further summarized as means ± standard deviations. Comparison of means was performed using paired sample t-test and Anova as appropriate while Pearson's test was used for correlation analysis. Results were presented in tables while statistical significance was set at $p<0.05$. Results: Fifty SCA patients were enrolled for the study and consisted of 26 (52%) females and 24 (48%) males. The overall mean age of the study participants was 22.82 ± 6.55 years with the minimum and maximum ages of 18 and 42 respectively. During the bone pain vaso-occlusive crises, 14 (28%), 21 (42%) and 15 (30%) of the patients had mild, moderate and severe bone pains respectively. The red blood cell parameters during the steady and bone pain crises states were RBC count (2.87±0.69 vs. 2.93±0.83; p=0.675); HCT (24.33±5.01 vs. .37±5.61; p=0.373); Hb concentration (8.34±1.53 vs. 7.99±1.54; p=0.228); MCV (87.87±9.13 vs. 82.59±14.64; p=0.021); MCH (29.76±5.61 vs. 30.48±13.54; p =0.729); MCHC (32.89±4.76 vs. 32.10±3.75; p=0.394) and RDW-CV (19.30±6.58 vs. 19.59±5.27; p=0.829) respectively. During bone pain vaso-occlusive crises, the red blood cell parameters based on mild, moderate and severe pains were RBC count (2.82±0.88 vs. 2.78±0.57 and 3.06±0.65; p=0.472); HCT (24.29±6.37 vs. 24.11±5.12 and 24.68±3.53; p=0.947); Hb concentration (8.46±2.01 vs. 8.33±1.57 and 8.24±0.92; p=0.927); MCV (87.60±7.46 vs. 91.03±9.21 and 83.69±9.22, p=0.055); MCH (31.28±7.77 vs. 30.27±4.27 and 27.61±4.51; p=0.185); MCHC (33.05±7.54 vs. 33.07±3.39 and 32.48±3.13; p=0.928) and RDW-CV (19.61±9.65 vs. 19.96±5.61 and 18.10±4.23; p=0.700) respectively. Conclusions: Of all the studied red cell parameters, only MCV significantly differ between the steady and bone pain crises states of SCA. A drop in MCV was recorded during the bone pain crises; probably reflecting depletion of the large reticulocytes and neocytes during the accentuated red cell sickling and vaso-occlusive events characteristic of this clinical state.

Keywords: Sickle cell anaemia, Bone pain crises, Red blood cell parameters, Red blood cell indices.

INTRODUCTION

Sickle cell anaemia (SCA) is an autosomal recessive haemoglobin disorder due to the inheritance of HbS in the homozygous state. It is the most common and severest form of sickle cell diseases arising from a point mutation (GAG→GTG) with resultant substitution of glutamic acid by valine at the sixth position of the β-globin chain [1, 2]. This substitution yields the HbS, which in conditions of low oxygen concentration, is less soluble when compared to HbA and thus crystallizes out of solution within the red cells. The cross-linking of the monomeric HbS molecules via the valine amino acids leads to polymer formation which elongates to form bundles and precipitate inside the red cells giving it various abnormal shapes including the 'sickle shape'. Repeated sickling if unabated may lead to permanent damage to the red cell membrane, vaso-occlusion and premature destruction of the red cells with gradual development of chronic anaemia [1-3]. SCA patients may be in a relative good health (steady state) which is periodically punctuated by periods of acute exacerbation or worsening of clinical features (crises state) [1-4]. The forms of crises include; vaso-occlusive (bone pain, acute chest syndrome, priapism, mesenteric infarction and stroke), aplastic and hyperhaemolytic crises [1-7].

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Consequent on the pathologic vaso-occlusive events and premature destruction of red cells encountered with SCA, red blood cell parameters such as the red blood cell count, haemoglobin concentration, haematocrit and the red cell indices of steady state SCA patients have been reported to differ from those of the non-SCA population and such alteration may add to our understanding of the pathophysiologic mechanisms of SCA and thus have bearing on the care of these patients [6, 8]. The worsening of red cell sickling and vaso-occlusion as often encountered during bone pain vaso-occlusive crises could further modify these parameters [6, 8].

The red cell parameters provide information on haemoglobin concentration as well as the quantity, sizes, shapes and physical characteristics of the red cells [9, 10]. Nowadays, these parameters are routinely determined via automation and utilized in the evaluation, diagnosis and classification of anaemia [9, 10].

Thus, the present study was carried out to determine the effect of bone pain vaso-occlusive crises on red cell parameters of patients with SCA.

METHODOLOGY

Study design, Study area and Study population:

This was a longitudinal study conducted on 50 SCA patients at the Department of Haematology and Blood Transfusion, UDUTH Sokoto, Nigeria between October 2019 and September 2020. Red blood cell parameters were established during the SCA steady states of the patient and same parameters were repeated when the patients presented while in bone pain crises.

Inclusion criteria:

Consenting adult SCA patients of 18 years of age and above were recruited for the study

Exclusion criteria:

Those patients with other forms of SCD (HbSC and HbSβthal), on hydroxyurea, recent use of drugs that could affect blood counts (antibiotics and steroids) and those with diabetes mellitus, systemic hypertension and renal disorders were excluded.

Definition of clinical variables:

i. Sickle cell anaemia (SCA): Diagnosis of SCA was established by HbSS pattern on haemoglobin electrophoresis (pH of 8.6) using cellulose acetate paper and finding of sickle cells on blood smear [5, 8, 11].

ii. Steady state SCA was defined as a period free of crisis extending from at least three weeks since the last clinical event and three months or more since the last blood transfusion to at least one week before the start of a new clinical event [4].

iii. Bone pain crises were defined as sudden onset of pain in the extremities, back, abdomen, chest or head that lasted at least 2 hours, led to a clinic visit, and could not be explained except by SCD; this definition excluded priapism, acute chest syndrome, right upper quadrant syndrome, osteomyelitis and strokes [7, 12].

iv. Severity of pain was ascertained using the numeric pain scale of zero to ten (0 represents no pain and 10 the worst pain). The scores were further grouped into mild 1-5, moderate 6-7 and severe 8-10 [7, 12].

Laboratory tests

Three millilitres of free flowing venous blood were collected from each study participant with 2.5mls dispensed into tri-potassium ethylene di-amine tetra-acetic acid (K_3-EDTA) anticoagulated container for red blood cell parameters' analysis and kept at room temperature until processing within 2 hours of collection. Red blood cell parameters were analysed via automation using Nortek 3-part differential haematology analyser which uses the principles of colorimetry for Hb estimation, impedance method for RBC count; while the HCT and the red cell indices were calculated by the analyser. A peripheral blood film stained with Leishmann stain was examined to cross-check the morphology of the red blood cells.

Reference values

The following reference values or ranges were utilized for interpretation of red blood cell parameters obtained during the study: RBC count 4.5-6.5 x 1012/l (adult male) and 3.8-5.8 x 1012/l (adult female); HCT 40-55% (adult male) and 38-48% (adult female); Hb 13-17g/dl (adult male) and 11.5-16.5 g/dl (adult female); MCV 76-96fL; MCH 27-32pg; MCHC 32-36g/dl; RDW-CV 11-16% [9].

Ethical Considerations

Approval to conduct this study was obtained from the Health and Ethics Research committees of Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, Nigeria.

STATISTICAL ANALYSIS

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM Corp, Armonk, NY, USA) and further summarized as means ± standard deviations. Comparison of means was performed using paired sample t-test and Anova as appropriate while Pearson's test was used for correlation analysis. Results were presented in tables while statistical significance was set at $p<0.05$.

RESULTS

A total of 50 SCA patients were enrolled for the study and consisted of 26 (52%) females and 24 (48%) males. The overall mean age of the study participants was 22.82 ± 6.55 years with the minimum and maximum ages of 18 and 42 respectively. During the bone pain vaso-occlusive crises, 14 (28%), 21 (42%) and 15 (30%) of the patients had mild, moderate and severe bone pains.

Table 1 shows the values of red cell parameters for the SCA patients during both steady and bone pain crises states. Table 1 also highlights the results of the comparison of the red cell parameters between the two clinical states of the patients. A drop in the mean HCT, Hb, MCV and MCHC were observed during the bone pain crises period; though this attained statistical significance only with respect to MCV. Furthermore, though the steady state period had lower mean RBC count, MCH and RDW none of these observation attained statistical significance.

Tables 2 and 3 depict the red blood cell parameters in both steady and bone pain crises states of the SCA patients based on gender. During the steady state, the females recorded higher mean HCT, Hb concentration, MCV, MCH, MCHCH and RDW but lower RBC count when compared with the males. However, with regards to the bone pain crises period, the females recorded higher mean values in respect of MCV, MCH and MCHC; while the RBC count, HCT, Hb and RDW had lower mean values. However, all the observed gender-based differences in both clinical states did not attain statistical significance ($P > 0.05$).

During the bone pain crises period, patients with severe bone pains recorded the lowest mean Hb and red cell indices (MCV, MCH, MCHC and RDW) but highest mean RBC count and HCT when compared to those with moderate and mild pains; however, these observations were not statistically significant ($P > 0.05$) as depicted in Table 4.

Study of correlation analysis revealed variety of relationships observed between the red cell parameters of the two clinical states; however,

none of these was found to be statistically significant (P > 0.05) as highlighted in Table 5.

Table 1: Comparison of red blood cell parameters between the steady and bone pain crises states

Parameters	Clinical state of patients		t-test	*p*- value
	Steady State n=50 Mean±SD	Bone Pain Crises n=50 Mean±SD		
*RBC count (X 10^{12}/l)	2.87±0.69	2.93±0.83	-0.421	0.675
†HCT (%)	24.33±5.01	23.37±5.61	0.898	0.373
‡Hb (g/dl)	8.34±1.53	7.99±1.54	1.220	0.228
$MCV (fL%)	87.87±9.13	82.59±14.64	2.379	0.021
||MCH (pg)	29.76±5.61	30.48±13.54	-0.349	0.729
¶MCHC (g/dl)	32.89±4.76	32.10±3.75	0.860	0.394
**RDW-CV (%)	19.30±6.58	19.59±5.27	-0.217	0.829

*RBC= red blood cell; †HCT=haematocrit; ‡Hb=haemoglobin; $MCV=mean corpuscular volume; ||MCH=mean corpuscular haemoglobin; ¶MCHC=mean corpuscular haemoglobin concentration; **RDW=red cell distribution width

Table 2: Red blood cell parameters during steady state based on gender

Parameter	Overall N=50 Mean±SD	Male n=24 Mean±SD	Female n= 26 Mean±SD	t-test	*P*- value
*RBC count (X 10^{12}/l)	2.87±0.69	2.90±0.85	2.85±0.52	0.291	0.773
†HCT (%)	24.33±5.01	24.12±5.53	24.53±4.58	-0.286	0.776
‡Hb (g/dl)	8.34±1.53	7.96±1.56	8.69±1.44	-1.713	0.093
$MCV (fL%)	87.87±9.13	85.65±9.26	89.91±8.68	-1.678	0.100
||MCH (pg)	29.76±5.61	28.49±5.60	30.92±5.47	-1.533	0.127
¶MCHC (g/dl)	32.89±4.76	32.65±5.27	33.12±4.33	-.340	0.735
**RDW-CV (%)	19.30±6.58	18.36±4.06	20.17±8.25	-.973	0.335

*RBC= red blood cell; †HCT=haematocrit; ‡Hb=haemoglobin; $MCV=mean corpuscular volume; ||MCH=mean corpuscular haemoglobin; ¶MCHC=mean corpuscular haemoglobin concentration; **RDW=red cell distribution width

Table 3: Red blood cell parameters during bone pain crises based on gender

Parameter	Overall N=50 Mean±SD	Male n=24 Mean±SD	Female n= 26 Mean±SD	t-test	*P*- value
*RBC count (X 10^{12}/l)	2.93±0.83	3.01±1.01	2.86±0.63	0.627	0.534
†HCT (%)	23.37±5.61	23.98±6.40	22.80±4.82	0.740	0.463
‡Hb (g/dl)	7.99±1.54	8.03±1.72	7.95±1.39	0.162	0.872
$MCV (fL%)	82.59±14.64	81.78±16.29	83.33±13.22	-0.371	0.712
||MCH (pg)	30.48±13.54	30.44±14.14	30.51±13.24	-0.017	0.896
¶MCHC (g/dl)	32.10±3.75	31.88±4.50	32.31±2.98	-0.408	0.685
**RDW-CV (%)	19.59±5.27	19.65±5.04	19.53±5.58	0.076	0.939

*RBC= red blood cell; †HCT=haematocrit; ‡Hb=haemoglobin; $MCV=mean corpuscular volume; ||MCH=mean corpuscular haemoglobin; ¶MCHC=mean corpuscular haemoglobin concentration; **RDW=red cell distribution width

Table 4: Comparison of red blood cell parameters during bone pain crises based on severity of pain

Parameter	Overall N=50 Mean±SD	Category of Participant based on Severity of Pain			F-test	*p*- value
		Mild Pain n=14 Mean±SD	Moderate Pain n= 21 Mean±SD	Severe Pain n= 15 Mean±SD		
*RBC count (X 10^{12}/l)	2.93±0.83	2.82±0.88	2.78±0.57	3.06±0.65	0.763	0.472
†HCT (%)	23.37±5.61	24.29±6.37	24.11±5.12	24.68±3.53	0.054	0.947
‡Hb (g/dl)	7.99±1.54	8.46±2.01	8.33±1.57	8.24±0.92	0.076	0.927
$MCV (fL%)	82.59±14.64	87.60±7.46	91.03±9.21	83.69±9.22	3.083	0.055
||MCH (pg)	30.48±13.54	31.28±7.77	30.27±4.27	27.61±4.51	1.750	0.185
¶MCHC (g/dl)	32.10±3.75	33.05±7.54	33.07±3.39	32.48±3.13	0.075	0.928
**RDW-CV (%)	19.59±5.27	19.61±9.65	19.96±5.61	18.10±4.23	0.359	0.700

*RBC= red blood cell; †HCT=haematocrit; ‡Hb=haemoglobin; $MCV=mean corpuscular volume; ||MCH=mean corpuscular haemoglobin; ¶MCHC=mean corpuscular haemoglobin concentration; **RDW=red cell distribution width

Table 5: Relationship between red blood cell parameters during steady state and bone pain crises

Parameters in steady state	Correlation analysis	Parameters during bone pain crises						
		*RBC count (X 10^{12}/l)	†HCT (%)	‡Hb (g/dl)	$MCV (fL%)	||MCH (pg)	¶MCHC (g/dl)	**RDW-CV (%)
*RBC count (X 10^{12}/l)	Spearman's r	0.078	-0.021	0.073	-0.115	-0.112	0.186	0.014
	p value	0.591	0.882	0.613	0.426	0.440	0.196	0.122
†HCT (%)	Spearman's r	0.022	-0.019	0.061	-0.090	-0.051	0.170	.017
	p value	0.882	0.898	0.672	0.536	0.727	0.238	0.907
‡Hb (g/dl)	Spearman's r	.033	0.063	0.114	-0.032	-0.026	0.049	-0.039
	p value	0.820	0.663	0.430	0.825	0.858	0.735	0.790
$MCV (fL%)	Spearman's r	-0.144	0.121	-0.053	0.192	0.043	-0.068	-0.160
	p value	0.319	0.403	0.713	0.181	0.768	0.640	0.267
||MCH (pg)	Spearman's r	-0.060	0.100	-0.036	0.130	0.013	-0.186	-0.101
	p value	0.681	0.481	0.805	0.369	0.930	0.195	0.486
¶MCHC (g/dl)	Spearman's r	0.136	0.043	0.089	-0.125	0.022	-0-137	0.100
	p value	0.346	0.767	0.538	0.387	0.881	0.342	0.488
**RDW-CV (%)	Spearman's r	-0.098	0.064	-0.033	0.176	-0.039	0.033	-0.206
	p value	0.500	0.656	0.819	0.220	0.788	0.822	0.151

*RBC= red blood cell; †HCT=haematocrit; ‡Hb=haemoglobin; $MCV=mean corpuscular volume; ||MCH=mean corpuscular haemoglobin; ¶MCHC=mean corpuscular haemoglobin concentration; **RDW=red cell distribution width

DISCUSSION

The hallmark of sickle cell anaemia (SCA) is the hypoxia-driven red cell sickling emanating from the presence of the abnormal HbS within the red cells. Repeated sickling if unabated may lead to permanent damage to the red cell membrane which predisposes to vaso-occlusion and premature destruction of the red cells either extravascular or within the blood vessels [1-6]. Thus, it is not surprising that we found our SCA patients (both steady and bone pain crises states) having lower values of RBC counts, haematocrit (HCT) and Hb concentration when compared with the reference values for the non-SCA adult black population [9]. Similar to our findings, several other researchers have reported lower mean values for the aforementioned red cell parameters amongst SCA patients when compared to normal reference ranges or HbAA control groups [14-19].

The red cell indices provide information on the morphological appearance and haemoglobin concentration of the red cells and have been useful in morphological classification and elucidating the aetiology of anaemia [9, 10].Though these indices could be calculated provided the values of red cell count, haemoglobin and haematocrit are known, they are nowadays routinely determined via automation. The mean corpuscular volume (MCV) defines the size of the red cell; the mean corpuscular haemoglobin (MCH) quantifies the amount of haemoglobin per red cell; the mean corpuscular haemoglobin concentration (MCHC) indicates the amount of haemoglobin per unit volume; and the red cell distribution width (RDW) represents the coefficient of variation of the red cell volume distribution (size)[9, 10]. Our study found the mean values for MCV, MCH and MCHC for both clinical states of the SCA within the reference range for the non-SCA population and thus giving a normocytic normochromic blood picture. In contrast to our finding, Akinbami *et al*., 2012 and Omoti *et al*., 2005 had reported higher mean MCV, MCH but lower MCHC for the steady state SCA when compared to their HbAA controls]. For SCA in crises state, Omoti *et al.*, 2005 [20] had observed higher mean MCV and MCHC but lower MCH when compared to the HbAA control. For the RDW, we observed higher mean value for the two clinical states of the SCA than that reported for non-SCA population; a finding in keeping with other studies and may not be unconnected to the haemolysis-induced bone marrow erythroid hyperplasia with consequent churning out into the peripheral circulation of younger cells in different stages of maturation and of different sizes [15, 20, 21].

The occurrence of bone pain crises which is a form of vaso-occlusive crises may further impact on red cell parameters and to this effect some studies have reported differences between the red cell parameters of the steady and crises states. This study found only MCV to significantly differ as it dropped with the development of bone pain

crises. This fall in MCV may be attributed to the utilization of the reticulocytes and other immature cells in the heightened red cell sickling and vaso-occlusion during bone pain crises. These younger cells are known to be larger in size and display abundance of cell-to-cell and cell-to-vascular adhesion molecules crucial for their active role in vaso-occlusion and their subsequent depletion [3, 5, 6, 8]. While, similar to our study, Abjah *et al*., 2017 [21] did not observe any significant difference between steady and crises states with respect to PCV; Abubakar *et al.,*2019 [22] found significant differences with regards to HCT and Hb concentration but not with MCV, MCH and MCHC. In the same vein, both Omoti *et al.,* 2005 and Antwi-Boasiako *et al.,* 2018 found differences in respect of Hb concentration, HCT, RBC count, MCV and RDW between the two clinical states [15, 20]. Some of the studies that reported higher mean Hb, MCV, MCH and MCHC during the crises state had postulated that during this state, blood viscosity is increased and that haemopoietic activity is much higher with the fresh red cells produced having higher indices [15, 20]. We also noted that severity of pain (for the bone pain crises state) and gender did not exert significant effect on the values of the red cell parameters. Both the separate studies by Abubakar *et al.*, 2019 and Iheanacho *et al.,* 2015 agreed with us in finding no gender-effect on these red cell parameters during the two clinical states [19, 21]. In contrast to this study, Antwi-Boasiako *et al.,* 2018 [15] found a gender effect during the crises state as they reported significant differences with RBC count, HCT, Hb concentration, MCV and RDW between the males and females.

It is pertinent to note that the variations recorded in red cell parameters amongst different SCA populations could arise due to nutritional factors (such as folate and iron) deficiency, co-existence of thalassaemia, βS haplotypes and genetic make-up of the patients [2, 19, 23]. Though this study did not screen for the presence of haematinic deficiency or thalassaemia, but its design (i.e. longitudinal study) enabled us to minimize effect of patient-related factors on red cell parameters; which many of the referenced studies, been largely cross-sectional in design, may not have addressed.

CONCLUSION

We observed normocytic normochromic anaemia in both steady and bone pain crises states of the studied SCA patients presumably due to the chronic haemolysis occasioned by the disease. With the development of bone pain crises, the only significant change recorded was a drop in MCV; probably reflecting utilization of the large and younger red cells (reticulocytes and neocytes) as red cell sickling and vaso-occlusive events get heightened during this vaso-occlusive state. Furthermore, our findings have underscored the need to consider individual-based values for red cell parameters in the management of SCA both in the steady and bone pain crises states.

Conflict of Interest

We declare that we have no conflict of interest

Author's Contribution

All authors substantially contributed to components of this study which included; conceptualization and design, data acquisition or analysis and interpretation, article drafting or critical revision as well as final draft approval.

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