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A comparative analysis of platelet parameters of sickle cell anemia patients during bone pain crises and in steady states

Abubakar Umar Musa, Mohammed A. Ndakotsu

Abstract:

BACKGROUND: Platelet parameters in steady-state sickle cell anemia (SCA) are affected by the red cell sickling, vaso-occlusion, and chronic hemolysis occasioned by the disease; and the occurrence of bone pain crises may further alter these parameters. Knowledge of platelet parameters in SCA augments our understanding of the pathophysiology of the disease and may influence disease management modalities.

OBJECTIVES: The objective of the study is to determine and compare platelet parameters of SCA patients during bone pain crises and in steady states.

PATIENTS AND METHODS: A longitudinal study involving 50 adult SCA patients who had platelet parameters determined during bone pain crises and later in steady states. Platelet count and platelet indices (mean platelet volume, platelet distribution width, plateletcrit, and platelet-large cell ratio (P-LCR)) were determined through automation.

RESULTS: SCA patients during both bone pain crises and steady states had higher mean platelet counts when compared with normal non-SCA reference values. P-LCR was found to be significantly different between the bone pain crises and steady states with mean values of 18.20 ± 5.55 versus 15.96 ± 4.91 respectively; $P = 0.034$. During the bone pain crises state, platelet parameters did not significantly differ based on the severity of pain.

CONCLUSIONS: Platelet count of both steady and bone pain crises states SCA patients were higher than the reference range for the normal non-SCA population. The P-LCR was the only platelet parameter that significantly differed between the two clinical states of SCA as it rose during the bone pain crises state; a finding reflecting increased peripheral platelet activation and the presence of larger circulating platelets during the vaso-occlusive crises.

Keywords:

Bone pain crises, platelet count, platelet indices, sickle cell anemia

Department of Haematology and Blood Transfusion, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria

Address for correspondence:
Dr. Abubakar Umar Musa, Department of Haematology and Blood Transfusion, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.
E-mail: abubakarumar38@yahoo.com

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Introduction

Higher platelet counts have been observed in steady-state sickle cell anemia (SCA) patients when compared to the normal non-SCA population due to anatomic and physiologic adaptation triggered by the chronic hemolysis and vaso-occlusion characteristic of the disease.[1,2] Additional findings are those of varying patterns of platelet indices such as increased mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT),[3] normal MPV but increased PDW.[1] The occurrence of bone pain vaso-occlusive crises may further alter these platelet parameters due to the attendant widespread endothelial damage and consequent platelet activation, aggregation, and consumption.[3-6] Platelet

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indices are markers of platelet activation and are related to the platelet's morphology and proliferation kinetics.[4] The platelet indices in common usage are determined through automation and include; MPV, PDW, PCT, and platelet-large cell ratio (P-LCR).[4] Researchers have shown the association between platelet indices and inflammatory processes that underlie a host of diseases including SCA.[5,6] Aside from prognostic and predictive significance, knowledge of pattern of platelet parameters could help tailor management modalities in the care of SCA patients.[5-7] Thus this study aimed to determine and compare platelet parameters of SCA patients (during bone pain crises and in steady states) attending a tertiary health-care facility in North-western Nigeria.

Patients and Methods

Study design, study area, and study population

This was a longitudinal comparative study which involved consecutive recruitment of 50 SCA patients receiving care between October 2019 and September 2020 at the Haematology Outpatient Clinic and Haematology Day Care Ward of the Department of Haematology and Blood Transfusion Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, Nigeria. The study participants had their platelet parameters determined first during the bone pain crisis state and a repeat of the same during the steady state.

Inclusion criteria

Consenting SCA patients of 18 years of age and above who presented at the study area during the study.

Exclusion criteria

Those excluded were other forms of sickle cell disease (SCD) such as hemoglobin SC (HBSC) and Hb S beta-thalassemia, patients on hydroxyurea, and recent use of drugs that could affect blood counts such as antibiotics, anticoagulants, and steroids. Furthermore, patients with medical diseases such as diabetes mellitus, systemic hypertension, and renal disorders were excluded from the study.

Definition of clinical variables

Sickle cell anemia

SCA was defined as a clinical disorder characterized by HbSS pattern on hemoglobin electrophoresis at alkaline pH of 8.6 using cellulose acetate paper and corroborated with the finding of sickle cells on Romanowsky stained peripheral blood smear.[8,9]

Steady state sickle cell anemia

Steady-state SCA was defined as a period with no history of acute painful episodes requiring hospitalization for the past 4 weeks, no history of blood transfusion in the previous 3 months, no history of intercurrent illness such as infection in the past 4 weeks, and no history of treatment with medications such as antibiotics that may affect the blood cell counts in the past 3 weeks.[10]

Bone pain crises sickle cell anemia

Bone pain crises were defined as sudden onset of pain in the extremities, back, abdomen, chest, or head that lasted at least 2 h, 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.[11]

Severity of pain

The severity of pain during bone crises was ascertained using the numeric pain scale of zero to ten (0 represents nil pain while 10 depicts the worst pain ever). The scores were further grouped into mild 1–5, moderate 6–7, and severe 8–10.[11]

Laboratory tests

During each of the crises and steady states, 3.5 mL of free-flowing venous blood were aseptically collected from study participants with 3.0 mL dispensed into a tri-potassium ethylene di-amine tetra-acetic acid anticoagulated container, adequately mixed and kept at room temperature for not more than 6 h before analysis.[12] Platelet counts and platelet indices: MPV, PDW, PCT, and P-LCR were determined through automation using Nortek hematology analyzer utilizing the impedance principle for blood cells count. Peripheral blood smears stained with Leishman's stain were examined to validate the platelet parameters.

The mean values of platelet parameters obtained were compared with established reference ranges for the normal non-SCA population [Appendix 1]. The following reference ranges were utilized: Platelet count 142–358 × 10^9/l; MPV 8.2–12.0 fl; PDW 9.4%–16.8%; PCT 0.17%–0.33%; and P-LCR 11.8%–37.4%.[13,14]

Ethical considerations

Approval to conduct the study was obtained from the Health and Ethics Research Committee UDUTH Sokoto, Nigeria.

Statistical analysis used

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM Corp, Armonk, NY, USA). The normality of data distribution was ascertained using Kolmogorov–Smirnov test and data were further summarized as means ± standard deviations (SDs). Comparison of means was performed using paired sample *t*-test and ANOVA as appropriate while the Spearman test was used for correlation analysis. Results were presented in tables and figures while statistical significance was set at $P < 0.05$.

Results

The mean ± SD age for the 50 SCA patients that participated in the study was 22.82 ± 6.55 years with the majority being females 26 (52%). Up to 21 (42%) of the study, participants had moderate pains during their bone pain crises states as depicted in Figure 1.

While none of the study participants had thrombocytopenia, the majority recorded thrombocytosis which was observed in 33 (66%) and 35 (70%) SCA patients during the bone crises and in the steady state, respectively.

Table 1 shows the values of platelet parameters for the SCA patients during both clinical states. It was observed that only the P-LCR was significantly different between the bone pain crises and the steady states with mean values of 18.20 ± 5.55 and 15.96 ± 4.91, respectively; (P = 0.034).

The pattern of the relationship of platelet parameters between the bone pain crises and steady states is depicted in Table 2; however, none of these relationships attained statistical significance.

A study of correlation analysis for the two SCA clinical states revealed a variety of relationships as shown in Tables 3 and 4. The platelet count had strong, positive, and significant relationship with PCT in both clinical states. While a negative relationship was recorded between the platelet count and P-LCR; this attained statistical significance only for the bone pain crises state (r = −0.322; P = 0.023). During both SCA clinical states, positive and significant relationships were recorded between the P-LCR and each of MPV and PDW.

Within the bone pain crises state, no significant differences were recorded amongst the patients based on the severity of their bone pains as depicted in Table 5.

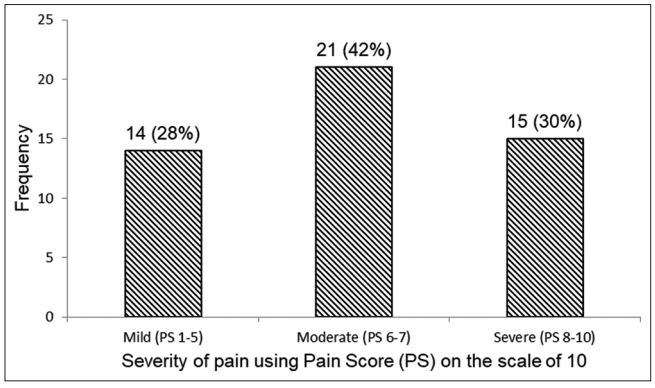

Figure 1: Distribution of participants based on severity of pain. PS = Pain score

Discussion

Our finding of higher mean platelet counts with the SCA patients during both the steady states and bone pain crises period when compared to the normal non-SCA adult population is in agreement with several other works.[2,3,15-21] This finding has been attributed to the hypoxia-driven increased erythropoietin production which induces the proliferation and maturation of megakaryocytes and the consequent observed increase in platelet production.[15] In addition, the occurrence of autosplenectomy in adult SCA patients due to repeated splenic infarctions reduces the splenic reservoir function and thus adds more platelet into the peripheral circulation.[15] We also observed thrombocytosis (platelet count >300 × 10^9/l)[22] in about two-third of our study participants (both in the steady state and bone pain crises) and this is similar to the finding of Ahmed *et al*.[15] who had reported thrombocytosis in all the 50 SCA patients (both in the steady state and vaso-occlusive crises) in their study. However, in contrast to our finding, Abjah *et al*.[23] reported normal platelet count for both steady state and crises to state SCA patients; and this observed difference could have resulted from variation in the composition of study participants based on the severity of pains in the addition to the effect of genetics and environment on hematological variables.[24] The occurrence of thrombocytosis and platelet activation in SCA patients has been shown to contribute to vascular occlusion which underlies much of the morbidity and mortality of the disease and thus measures instituted toward identifying and addressing such occurrences will go a long way in averting SCA-related complications.[5-7,25]

The red cell sickling in SCA and the eventual vaso-occlusions that ensue lead to widespread vascular endothelial injury and exposure to subendothelial matrix which may trigger platelets' activation, adhesion, aggregation, and consumption in the process of hemostasis.[5,26] This may largely explain our finding of a slight drop in mean platelet count during bone pain vaso-occlusive crises when compared to the steady

Table 1: Comparison of platelet parameters of study participants in the steady state and during bone pain crises (*n*=50)

Parameters	Participants (mean±SD)		*t*-test	*P*
	Steady state	Bone pain crises		
PC (×10^9/L)	438.78±224.33	374.46±129.52	1.717	0.092
MPV (fL)	8.01±0.0.72	9.60±9.32	−1.225	0.226
PDW (%)	13.54±1.58	14.23±1.77	−2.015	0.049
PCT (%)	0.34±0.17	0.31±0.11	1.261	0.213
P-LCR (%)	15.96±4.91	18.20±5.55	−2.186	0.034

PC=Platelet count, MPV=Mean platelet volume, PDW=Platelet distribution width, PCT=Plateletcrit, P-LCR=Platelet-large cell ratio, SD=Standard deviation

Table 2: Relationship between platelet parameters of study participants in the steady state and during bone pain crises

Parameters in steady state	Correlation analysis	Parameters during bone pain crises				
		PC (×10⁹/L)	MPV (fL)	PDW (%)	PCT (%)	P-LCR (%)
PC (×10⁹/L)	Spearman's *r*	−0.031	0.150	0.115	0.002	0.122
	P	0.831	0.299	0.428	0.989	0.399
MPV (fL)	Spearman's *r*	−0.078	0.163	0.173	−0.030	0.138
	P	0.059	0.258	0.228	0.838	0.340
PDW (%)	Spearman's *r*	0.091	0.020	−0.070	0.104	0.054
	P	0.531	0.890	0.627	0.471	0.712
PCT (%)	Spearman's *r*	−0.034	0.186	0.161	0.021	0.159
	P	0.817	0.196	0.263	0.883	0.271
P-LCR (%)	Spearman's *r*	−0.011	0.110	0.105	0.017	0.065
	P	0.939	0.446	0.468	0.907	0.655

PC=Platelet count, MPV=Mean platelet volume, PDW=Platelet distribution width, PCT=Plateletcrit, P-LCR=Platelet-large cell ratio

Table 3: Relationship between platelet parameters of study participants in the steady state

Parameter	Correlation analysis	Parameter				
		PC (×10⁹/L)	MPV (fL)	PDW (%)	PCT (%)	P-LCR (%)
PC (×10⁹/L)	Spearman's *r*	-	−0.035	−0.286	0.974	−0.219
	P	-	0.810	0.044	0.000	0.126
MPV (fL)	Spearman's *r*	−0.035	-	0.612	0.126	0.933
	P	0.810	-	0.000	0.382	0.000
PDW (%)	Spearman's *r*	−0.286	0.612	-	−0.201	0.617
	P	0.045	0.000	-	0.161	0.000
PCT (%)	Spearman's *r*	0.974	0.126	−0.201	-	−0.069
	P	0.000	0.382	0.161	-	0.635
P-LCR (%)	Spearman's *r*	−0.219	0.933	0.617	−0.069	-
	P	0.126	0.000	0.000	0.635	-

PC=Platelet count, MPV=Mean platelet volume, PDW=Platelet distribution width, PCT=Plateletcrit, P-LCR=Platelet-large cell ratio

Table 4: Relationship between platelet parameters of study participants during bone pain crises

Parameter	Correlation analysis	Parameter				
		PC (×10⁹/L)	MPV (fL)	PDW (%)	PCT (%)	P-LCR (%)
PC (×10⁹/L)	Spearman's *r*	-	−0.118	−0.541	0.955	−0.322
	P	-	0.415	0.000	0.000	0.023
MPV (fL)	Spearman's *r*	−0.118	-	0.332	0.051	0.786
	P	0.415	-	0.018	0.724	0.000
PDW (%)	Spearman's *r*	−0.541	0.332	-	−0.502	0.465
	P	0.000	0.018	-	0.000	0.001
PCT (%)	Spearman's *r*	0.955	0.051	−0.502	-	−0.154
	P	0.000	0.724	0.000	-	0.286
P-LCR (%)	Spearman's *r*	−0.322	0.786	0.465	−0.154	-
	P	0.023	0.000	0.001	0.286	-

PC=Platelet count, MPV=Mean platelet volume, PDW=Platelet distribution width, PCT=Plateletcrit, P-LCR=Platelet-large cell ratio

state and is in agreement with the separate studies of Ahmed *et al.* and Antwi-Boasiako *et al.* from Nigeria and Ghana respectively.[3,15] In contrast, Omoti[20] had reported a rise in platelet count during vaso-occlusive crises. Furthermore, our finding of further drop in platelet count, although not statistically significant, as the severity of bone pain worsened probably reflects the intensity of the widespread vascular endothelial injury as the vaso-occlusion progresses. However, It is pertinent to note that despite the observed drop in platelet count during the bone pain vaso-occlusive crises, no patient had thrombocytopaenia (platelet count<100 × 10^9/l);[22] reflecting a modest reduction in the platelet count as documented by the earlier works of Ahmed *et al*.[15]

With regards to the studied platelet indices, the MPV gives the average size of platelets in peripheral circulation with higher values occurring in disorders having peripheral destruction of platelets; thus, young platelets become bigger and increase in activity, whereas lower values indicate low production of platelets.[4,16] The PDW measures variability in the size distribution of platelets

Table 5: Comparison of platelet parameters of participants during bone pain crises based on severity of pain

Parameter	Mean±SD				*P*##
	Steady state (*n*=50)	Category of participant based on severity of pain			
		Mild pain (*n*=14)	Moderate pain (*n*=21)	Severe pain (*n*=15)	
PC (×10⁹/L)	438.78±224.33	397.64±112.38	388.29±136.85	333.47±132.69	0.341
MPV (fL)	8.01±0.0.72	8.29±0.51	11.31±14.38	8.41±0.88	0.551
PDW (%)	13.54±1.58	14.25±1.08	13.99±1.54	14.56±2.5	0.645
PCT (%)	0.34±0.17	0.33±0.91	0.31±0.10	0.29±1.30	0.637
P-LCR (%)	15.96±4.91	17.96±5.10	17.54±5.76	19.35±5.86	0.625

##ANOVA test. PC=Platelet count, MPV=Mean platelet volume, PDW=Platelet distribution width, PCT=Plateletcrit, P-LCR=Platelet-large cell ratio, SD=Standard deviation

and increases on platelet activation with higher values reported with megakaryocyte hyperplasia secondary to increased peripheral platelet consumption.[4] The P-LCR gives in percentage all circulating platelets with a volume measuring >12fL (usually large and young platelets) and has a direct relationship with MPV and PDW but an inverse relation with platelet count.[4] The PCT measures the total platelet mass as a percentage of the volume occupied in the blood. The PCT correlates well with the platelet count and indicates comparable clinical implications.[4] Our observations of a positive relationship between platelet count and PCT; and an inverse one between the platelet count and MPV, PDW, and P-LCR during both the steady and bone pain crises are in keeping with the platelet's morphology and proliferation kinetics typical of a chronic hemolytic disorder such as SCA.[4,16] Thus, our findings of slightly higher mean MPV, PDW, and P-LCR against the backdrop of lower mean platelet count and PCT during the bone pain vaso-occlusive crises when compared to the steady state strongly support the understanding that "vaso-occlusive crises" are associated with peripheral activation and destruction of platelets with consequent mobilization of younger and larger platelets from the bone marrow and into the peripheral circulation.[4-7]

Limitations

The limitations of this study include the noninclusion of age- and sex-matched normal HbAA individuals to serve as a control or additional comparison group.

Conclusions

Our study concluded that while the mean platelet counts of SCA patients (both steady and bone pain crises state) were higher when compared with the reference ranges for non-SCA populations; the platelet indices did not differ between the two populations. We also found P-LCR as the only platelet parameter that significantly differ between the two SCA clinical states as its mean value rose during the bone pain crises state; a finding reflecting increased peripheral platelet activation and the presence of larger circulating platelets during vaso-occlusive crises.

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Conflicts of interest

There are no conflicts of interest.

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Appendix 1: Comparing mean and standard deviation of platelet parameters with published reference ranges

Parameter	State of SCA	Mean±SD	Minimum	Maximum	Reference ranges for normal population	
					Abbas *et al*., (2016)[13]	Ken-Ezihu *et al*., (2017)[14]
PC ($\times 10^9$/L)	Steady	438.78±224.33	135	1220	146–378	142–358
	Bone pain crises	374.46±129.52	139	630		
MPV (fL)	Steady	8.01±0.72	6.3	9.3	8.2–11.6	8.2–12.0
	Bone pain crises	9.60±9.32	6.8	74.0		
PDW (%)	Steady	13.54±1.58	8.9	19.6	8.3–15.9	9.4–16.8
	Bone pain crises	14.23±1.77	10.4	21.8		
PCT (%)	Steady	0.34±0.17	0.12	0.97	0.13–0.34	0.17–0.33
	Bone pain crises	0.31±0.11	0.10	0.50		
P-LCR (%)	Steady	15.96±4.91	4.9	24.7	11.8–37.4	-
	Bone pain crises	18.20±5.55	8.6	33.9		

PC=Platelet count, MPV=Mean platelet volume, PDW=Platelet distribution width, PCT=Plateletcrit, P-LCR=Platelet-large cell ratio, SD=Standard deviation, SCA=Sickle cell anemia