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Evaluation of Changes in Haematological Parameters of Sickle Cell Anaemia Subjects in Rivers and Bayelsa States

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Authors' contributions

This work was carried out in collaboration among all authors. Author EME designed the study. Author SOA wrote the protocol. Author ACUE wrote the first draft of the manuscript. Author BSM managed the analyses of the study. Author NCI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to evaluate changes in some haematological parameters in sickle cell anaemia subjects in Rivers State.
Study Design: This study is a cross-sectional observational study.
Place and Duration of Study: University of Port Harcourt Teaching Hospital, Rivers State, and the Federal Medical Centre, Yenagoa, Bayelsa State, between the months of February and August, 2020.
Methodology: A total of four hundred and fifty (450) subjects with age range of 1-50 years were randomly selected. There are about 200 registered patients (adults and children alike) at the sickle cell clinics of the University of Port Harcourt Teaching Hospital, and the Federal Medical Centre,

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Yenagoa, with an average of 4 new patients per month. The sample size was obtained using a prevalence of sickle cell anaemia of 2% and the sample size was calculated using Cochran sample size formula. Five milliliters (5ml) of venous blood sample was withdrawn from the peripheral vein in the upper limb of subjects using a standard venipuncture technique. The sample was rocked gently to mix and kept at room temperature and the haematological parameters were analyzed within 4 hours of samples collection. The haematological parameters: total white blood cell count (WBC), red blood cell count (RBC), Haemoglobin concentration (Hb), Haematocrit, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW-CV), Platelet count (PLT), MPV, Neutrophils, Lymphocyte, Monocyte, Eosinophils, and Basophils) were analyzed using Mindray BC-6800 auto Haematology analyzer system. Data management and statistical analyses were conducted using Statistical Analyses System SAS 9.4 (SAS Institute, Cary, North Carolina, USA) and p values less than *.05* were considered statistically significant.

Results: The results showed the mean comparison of haematological parameters in sickle cell anaemia and control subjects. The mean comparison of Haemoglobin F was significantly reduced statistically (*p<.05*) in vaso-occlusive crises (VOC) condition than steady state compared with the control group. There was increase trend of haematological parameters showing statistically significant difference across the subject conditions compared with the control. There were exceptions in few cases especially in lymphocytes which was not significant (*p>.05*) in the steady state and vaso-occlusive crisis compared with the control. Similarly, Neutrophils was not significant (*p>.05*) in the steady state and vaso-occlusive crisis compared with the control. Furthermore, Basophils was more significant (*p<.05*) in the steady state than in the vaso-occlusive crisis and control groups. Similarly, absolute eosinophil was less significant statistically (*p<.05*) in the steady state and vaso-occlusive crisis than in the control group.

Conclusion: This study has shown that there are changes in haemtological parameters between SCA subjects and control subjects and the VOC and steady state sickle cell anaemia (SCA) subjects.

Keywords: Evaluation; haematological parameters; sickle cell anaemia; Rivers State.

1. INTRODUCTION

Despite being a monogenic disorder, sickle cell anaemia (SCA) presents with extreme phenotypic variability [1]. Haemolytic anaemia, vaso-occlusion and vasculopathy are the hallmarks of SCA pathophysiology, but it is now clear that multiple actors including leukocytes, platelets, endothelial cells, pro-inflammatory cytokines, oxidative stress and reduced nitric oxide (NO) availability, and haemostatic activation play a role in disease expression [1-2]. According to global estimates, approximately 5% of the population has some type of haemoglobin variant, and more than 300,000 babies are born each year with haemoglobinopathies, with the homozygous sickle cell disease (HbSS) being the most prevalent type [3-5].

It is estimated that the prevalence of live births with the disease is 4.4% in the world, where rates remain high on the main continents of Africa, Southeast Asia, and the Americas [5]. However, worrying estimates indicate that the number of newborns with SCA will increase from approximately 300,000 in 2010 to 400,000 in 2050 [6-7]. The African continent, which has 3.6 million new cases of sickle cell trait (HbAS) and 238,000 SCA, remains the largest cradle of SCA genetic inheritance [8-9] with a 3% and 2% prevalence of HbSS in Rivers and Bayelsa States respectively, both in South-Southern Nigeria [10-11]. Nigeria, and the Democratic Republic of Congo would urgently need to plan policies for prevention and management of SCA, so that implementations carried out in 2015 could save many lives by 2050 [11].

The pathological presentation of SCA begins with the process of formation of Haemoglobin S (HbS) polymers which triggers dehydration and increased cell stiffness, giving rise to the vaso-occlusion event [12-13]. This phenomenon leads to the appearance of several pathophysiological events such as tissue ischemia, anaemia, inflammation, and haemolysis [14-15]. The actual anaemia of the illness is caused by haemolysis, the destruction of the red cells, because of their shape [16]. Although the bone marrow attempts to compensate by creating new red cells, it does not match the rate of destruction**.** Healthy red blood cells typically function for 90–120 days, but sickled cells only last 10–20 days [17].

It is known that under hypoxic conditions, deoxygenation triggers a hydrophobic interaction between the mutated haemoglobin (HbS) molecules, resulting in the polymerization of HbS and sickling of the RBCs. Sickling alters the cell membrane properties, which reduce cellular flexibility and lead to unusual cell adherence to vascular endothelium [18]. Studies further suggest that sickling alters the RBCs' membrane properties including a significant expression/exposure of different adhesive molecules, which mediate the adhesion of sickle RBCs to the endothelium and subendothelial matrix [19].

Basically, sickle RBCs express a significant quantity of phosphatidylserine (PS) on the cell surface and promote the adhesion to the endothelium through binding to $\alpha_{II}\beta_{III}$ [20]. Besides the increase of expression/exposure of adhesive molecules on sickle RBCs, which is associated with increased cell adhesion to the vessel wall, sickling also causes the release of excessive extracellular haemoglobin (ECHb) into plasma from the sickle RBCs during intravascular haemolysis. Therefore, the aim of this study was to evaluate changes in some haematological parameters in sickle cell anaemia subjects in Rivers State.

2. MATERIALS AND METHODS

2.1 Study Design

This study is a cross-sectional study. Four hundred and fifty (450) subjects were grouped into three as follows: Group 1 (150 sickle cell anaemia subjects at steady that were clinically defined as free of infection, pain or any evidence of active disease at least 2 weeks prior to the next clinical visit and three months after blood transfusion) [21], Group 2 (150 sickle cell anaemia subjects with vaso-occlusive crises which were clinically defined of having pain in the bones, muscles, and joints not attributable to any other cause and requiring parenteral analgesic and hospitalization at the hospital for some hours) [21-22] and finally Group 3 (150 apparently healthy individuals with homozygous haemoglobin A genotype who served as the control subjects).

2.2 Study Area

The study was carried out in two Federal tertiary hospitals, the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt and Federal Medical Centre (FMC) Yenagoa, both in Nigeria. The two Federal hospitals were formerly located in the same state (old Rivers State). They are referral centres for various health institutions in the respective states, Rivers and Bayelsa, Nigeria.

2.3 Study Population

A total of three hundred (300) subjects with age range of 1-50 years were randomly selected. There are about 200 registered patients (adults and children alike) at the sickle cell clinics of the University of Port Harcourt Teaching Hospital, and the Federal Medical Centre, Yenagoa, with an average of 4 new patients per month. The clinics run on Thursdays and Fridays respectively with a weekly attendance of between five (5) and ten (10) patients. It was runned by two (2) Consultants, two (2) Senior Registrars, and three (3) Registrars.

2.4 Eligibility Criteria

2.4.1 Inclusion criteria

Subjects with homozygous haemoglobin S (HbSS) aged between 1 year and above who had been apparently well with no recent drop in the haemoglobin level and there was absence of infection, pain, acute complicating factors or acute clinical symptoms or crisis for a minimum of two (2) weeks before recruitment as established by a careful history and complete physical examination [23,22]. Subjects with homozygous haemoglobin S (HbSS) aged one year and above who had crises, also referred to the episodes of acute illness attributable to the sickling phenomenon in which there was a sudden exacerbation of symptoms and signs of subjects who had hitherto been in stable condition. The pain was in the form of vaso-occlusive crisis, aplastic crisis, acute sequestration crisis, or haemolytic crises [23,22].

2.4.2 Exclusion criteria

Subjects who non-homozygous genotype (Hb SS) were excluded from the study. Also, subjects with any type of infective illness (HIV, tuberculosis, SARS-COV 19) or has had recent blood transfusion during the preceding two weeks were excluded from the study. Furthermore, subjects who had recent intake of any myelosuppressive agent (e.g., Hydroxyurea) for the preceding two weeks were excluded.

2.5 Sample Size

Purposive sampling and randomized method were used in the selection of subjects, with due consideration of the total number of patients admitted in the clinic/ward in the University of Port Harcourt Teaching Hospital, Port Harcourt and the Federal Medical Centre, Yenagoa. The sample size was obtained using a prevalence of sickle cell anaemia of 2% [10] and the sample size was calculated using Cochran sample size formula [24].

$$N = \frac{Z^2 pq}{d^2}$$

Where

N =the desired sample size
Z = the Standard Normal deviate usually set at 1.96 corresponding to the 95% Confidence level
p = the prevalence of target population
q = 1- p
d = degree of accuracy desired set at 0.05

$$\text{Therefore } N = \frac{(1.96)^2 \times 0.02 \times (1-0.02)}{(0.05)^2}$$

p = % or 0.02
N = 30
By adding 10% of non-respondent, the sample size will be 33.

2.6 Sample Collection and Handling

Five milliliters (5 ml) of venous blood sample was withdrawn from the peripheral vein in the upper limb of subjects using a standard venipuncture technique with minimum stasis under aseptic conditions from the dorsum of the hand or ante-cubital vein as the case may be as described by Ian in 2017 [25]. This was done by fastening a soft tourniquet to help locate and define peripheral veins to achieve successful and safe venipuncture, not more than 2 minutes to enable the index finger feel a suitable vein. The punctured site was then cleaned with 70% alcohol (methylated spirit) before collection of samples with 21G dry sterile hypodermic needle with a 5ml syringe, thereafter, 2ml of the whole blood was transferred into EDTA bottle.

The sample was rocked gently to mix and kept at room temperature and the haematological parameters were analyzed within 4 hours of samples collection. The separated plasma was stored at -20^{o}C prior to assay. Assay was carried out on the plasma sample thawed only once. The sample bottles were then assigned a study code with a non-water-soluble ink with date, sex and time of collection and logged on to a paper log after dispensing the blood sample into the sample bottles.

2.7 Laboratory Estimations

2.7.1 Estimation of haematological parameters

The haematological parameters, total white blood cell count (WBC), red blood cell count (RBC), Haemoglobin concentration (Hb), Haematocrit, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW-CV), Platelet count (PLT), MPV, Neutrophil absolute-#, Neutrophil %, Lymphocyte absolute-#, Lymphocyte %, Monocyte absolute-#, Monocyte %, Eosinophil absolute-#, Eosinophil %, Basophil absolute-#, and Basophil %) were analyzed using Mindray BC-6800, an auto Haematology analyzer system, [26]. This is based on a combination of light scatter, electrical impedance, fluorescence, light absorption, and electrical conductivity methods to produce complete red blood cell, platelet, and leukocyte analyses. All the widely used automated instruments analyze cell in flow and are essential highly specialized flow cytometers.

2.7.1.1 Quality control of full blood count determination

Appropriate volume of blood and anticoagulant was used in order to maintain the specimen's quality. In addition, the autoanalyzer has an automatic in-built control program that ensured accuracy in the events that passed though the laser for better result output. As the analyser was powered from the direct circuit (DC) button, the main unit was turned on, which eventually entered an auto rinse mode in which the instrument was rinsed three to five times, waste was drained, background counts were reviewed and the electronics, injector piston motors, bubble memory, and instrument status were automatically checked. (An auto rinse switch is available to the operator for a background check whenever desired). Manually prepared blood films were viewed for clot formation and PCV results were checked against haematocrit results.

2.8 Statistical Analysis

Data management and statistical analyses were conducted using Statistical Analyses System SAS 9.4 (SAS Institute, Cary, North Carolina, USA). The mean/standard deviation and Analysis of Variance (ANOVA) with interaction effects where significant differences existed in the subject states or interaction of the states with any of the independent factors were determined. Post Hoc Tests was conducted using the Tukey's Honestly Significant Difference (HSD) procedure to compare all possible pairs of the parameters means. Pearson Correlation analysis was used to determine the direction of relationships within and between the haemtological parameters by the sickle cell anaemia subjects' state.

3. RESULTS AND DISCUSSION

This study showed that the mean comparison of haematological parameters in sickle cell anaemia as compared with the control subjects significantly decreased statistically (*p<.05*) across the subject states compared with the control. The total WBC count observed in this study was significantly higher among subjects with SCA than the controls. Same observation was made by Antwi-Baffour and colleagues in 2019 [27], Hilliard and colleagues in 2018 [28] and Kusfa and colleagues in 2017 [29].

Separately, in previous studies, leukocytosis was found to be a common feature in patients with SCA in steady state in the absence of infection. In another study by Kusfa and colleagues [29] who suggested that the generation of an inert inflammatory response led to release of cytokine mediators whose function is to increase production of neutrophils by the bone marrow. This agrees with studies carried out by other researchers that sickle cell anaemia (SCA) is often characterized by marked inflammation, leukocytosis, leukocyte activation, and potentially increased leukocyte adhering to the vascular endothelium [30].

This leukocyte adhesion to the endothelium could itself promote vaso-occlusion, [31] which is the hallmark of SCA [32]. A previous report by Ahmed and colleagues in 2017 [33] and Okpala in 2004 [30] indicated that SCA subjects have elevated white blood cell (WBC) counts activated granulocytes, monocytes, and endothelial cells, enhanced expression of endothelial cell adhesion molecules, elevated cytokine levels and elevated acute-phase reactants [31]. A contrary report by Turhan and colleagues in 2012 [34] showed no significant difference in the total leukocyte count between the patients with SCA in steady state and that of the controls, even though the study was carried out in children. This finding may depend on maternal factors as maternal health, nutritional status, and antenatal complication such as anaemia.

Table 1. Comparison of haematological parameters in sickle cell anaemia and control subjects

Parameter	Subject state			Test Statistics	
	Steady State (n=150)	Vaso-Occlusive Crisis (VOC) (n=150)	Control (n=150)		
	Mean ± SD	Mean ± SD	Mean ± SD	F-Ratio	Prob > F
HbF (%)	7.99±0.218 [a]	0.68±0.038 [b]	1.18±0.027 [c]	1006.179	<0.05*
HCT (%)	30.30±0.381 [a]	22.26±0.429 [b]	42.21±0.333 [c]	686.918	<0.05*
HB (g/dL)	9.70±0.136 [a]	7.73±0.159 [b]	14.03±0.119 [c]	536.278	<0.05*
RBC (x10^12)	3.82±0.089 [a]	2.92±0.137 [b]	4.95±0.086 [c]	91.965	<0.05*
WBC (x10^3/µL)	7.35±0.357 [a]	13.15±0.696 [b]	5.22±0.122 [c]	80.633	<0.05*
MCV (fl)	74.74±0.860 [a]	76.27±0.988 [a]	85.27±0.555 [b]	48.032	<0.05*
MCH (pg)	26.78±0.319 [a]	24.17±0.360 [b]	31.16±0.184 [c]	141.154	<0.05*
MCHC (g/dL)	31.09±0.277 [a]	30.55±0.293 [a]	33.92±0.130 [b]	54.830	<0.05*
RDW-CV (%)	18.67±0.439 [a]	19.13±0.300 [a]	14.01±0.137 [b]	79.590	<0.05*
LYMPH	4.69±0.180 [a]	7.84±0.246 [b]	1.44±0.046 [c]	17.622	<0.05*
NEUT	4.33±0.245 [a]	9.11±0.400 [b]	2.95±0.127 [c]	37.778	<0.05*
EOSIN	1.144±0.016 [a]	3.274±0.032 [b]	0.215±0.011 [c]	9.155	<0.05*
MONO	0.513±0.032 [a]	1.502±0.276 [b]	0.466±0.013 [a]	13.239	<0.05*
BASO	0.050±0.006 [a]	0.077±0.006 [b]	0.043±0.004 [a]	11.528	<0.05*
PLT (x10^3/µL)	191.23±5.816 [a]	146.27±7.117 [b]	256.81±6.199 [c]	75.437	<0.05*
MPV (fL)	7.61±0.194 [a]	7.56±0.277 [a]	10.84±0.085 [b]	87.567	<0.05*

*Within parameters, means ± SD across subjects' states with different superscripts (a, b, c, ab) are significantly different at p<.05. Significance Levels: *=significant (p<.05), ns=not significant (p>.05)*

Table 2a. Pairwise correlations of hematological parameters by sickle cell anaemia subjects' state

Parameter	By Parameter	Subjects state					
		Steady state (n=150)		Vaso-occlusive crisis (VOC) (n=150)		Control (n=150)	
		Correlation	P-Value	Correlation	P-Value	Correlation	P-Value
HB (g/dL)	HCT (%)	0.815	<0.0001*	0.772	<0.0001*	0.158	0.0531
RBC (x10^12/µL	HCT (%)	0.353	<0.0001*	0.224	0.0060**	0.069	0.4003
RBC (x10^12/µL	HB (g/dL)	0.241	0.0030*	0.326	<0.0001*	-0.129	0.1159
WBC (x10^3/µL)	HCT (%)	-0.019	0.8174	-0.322	<.0001*	0.033	0.6864
WBC (x10^3/µL)	HB (g/dL)	0.078	0.3433	-0.344	<0.0001*	0.065	0.4271
WBC (x10^3/µL)	RBC (x10^12/µL	-0.086	0.2965	-0.099	0.2259	0.174	0.0335*
MCV (fl)	WBC (x10^3/µL)	-0.090	0.2726	0.175	0.0324*	0.158	0.0533
MCH (pg)	RBC (x10^12/µL	-0.134	0.1033	-0.106	0.1961	-0.177	0.0300*
MCH (pg)	MCV (fl)	0.163	0.0465*	0.491	<0.0001*	-0.094	0.2549
MCHC (g/dL)	HCT (%)	0.164	0.0456*	0.152	0.0646	0.034	0.6828
MCHC (g/dL)	HB (g/dL)	0.144	0.0790	0.187	0.0226*	0.027	0.7460
MCHC (g/dL)	RBC (x10^12/µL	-0.178	0.0302*	0.129	0.1160	-0.066	0.4240
MCHC (g/dL)	MCH (pg)	-0.182	0.0266*	0.285	0.0004*	0.015	0.8597
RDW-CV (%)	HCT (%)	-0.113	0.1697	-0.207	0.0109*	0.097	0.2388
RDW-CV (%)	HB (g/dL)	-0.070	0.3976	-0.228	0.0049*	-0.085	0.3039
RDW-CV (%)	MCV (fl)	-0.142	0.0824	0.062	0.4523	0.192	0.0184*
LYMPH	HCT (%)	0.051	0.5378	-0.272	0.0008*	0.043	0.6010
LYMPH	HB (g/dL)	0.114	0.1647	-0.237	0.0035*	0.167	0.0409*
LYMPH	RBC (x10^12/µL	-0.170	0.0371*	-0.153	0.0624	-0.102	0.2122
LYMPH	WBC (x10^3/µL)	0.476	<0.0001*	0.545	<0.0001*	-0.022	0.7923
LYMPH	MCH (pg)	-0.170	0.0378*	0.069	0.4016	0.175	0.0326*
LYMPH	MCHC (g/dL)	0.204	0.0126*	0.118	0.1533	-0.103	0.2112
LYMPH	RDW-CV (%)	0.028	0.7345	0.035	0.6747	-0.163	0.0457*

Significance Level:= p<0.05*

Table 2b. Pairwise correlations of hematological parameters by sickle cell anaemia subjects' state

Parameter	By parameter	Subjects state					
		Steady state (n=150)		Vaso-occlusive crisis (VOC) (n=150)		Control (n=150)	
		Correlation	P-Value	Correlation	P-Value	Correlation	P-Value
NEUT	RBC (x10^12/µL	0.028	0.7347	-0.140	0.0873	-0.191	0.0192*
NEUT	WBC (x10^3/µL)	0.746	<0.0001*	0.592	<0.0001*	-0.081	0.3268
NEUT	MCV (fl)	-0.054	0.5124	0.202	0.0130**	0.131	0.1097
NEUT	MCH (pg)	0.032	0.6969	0.165	0.0443*	0.089	0.2794
NEUT	Abs. LYMPH	0.163	0.0467*	0.217	0.0077*	-0.102	0.2124
NEUT	LYMPH (%)	-0.475	<0.0001*	-0.610	<0.0001*	0.033	0.6895
EOSIN	HCT (%)	-0.038	0.6402	-0.121	0.1402	0.094	0.2524
EOSIN	WBC(x10^3/µL)	0.401	<0.0001*	0.229	0.0049*	-0.010	0.9072
EOSIN	MCHC (g/dL)	0.167	0.0415*	0.107	0.1947	-0.038	0.6463
EOSIN	Abs. LYMPH	0.373	<0.0001*	0.451	<0.0001*	-0.031	0.7038
EOSIN	LYMPH (%)	-0.120	0.1440	-0.182	0.0261*	-0.098	0.2329
EOSIN	Abs. NEUT	0.390	<0.0001*	0.011	0.8978	0.147	0.0729
EOSIN	NEUT (%)	-0.084	0.3041	-0.028	0.7315	0.162	0.0470*
MONO	HCT (%)	-0.024	0.7741	-0.172	0.0350*	0.096	0.2430
MONO	HB (g/dL)	0.011	0.8984	-0.184	0.0239*	0.034	0.6798
MONO	WBC(x10^3/µL)	0.584	<0.0001*	0.620	<0.0001*	-0.018	0.8231
MONO	MCV (fl)	-0.022	0.7894	-0.190	0.0201*	-0.054	0.5113
MONO	MCHC (g/dL)	0.190	0.0204*	0.132	0.1088	-0.037	0.6544
MONO	RDW-CV (%)	0.079	0.3388	-0.061	0.4614	0.034	0.6794
MONO	Abs. LYMPH	0.570	<0.0001*	0.521	<0.0001*	-0.018	0.8307

Significance Level:= p<0.05*

Table 2c. Pairwise correlations of hematological parameters by sickle cell anaemia subjects' state

Parameter	By Parameter	Subjects State					
		Steady state (n=150)		Vaso-occlusive crisis (VOC) (n=150)		Control (n=150)	
		Correlation	P-Value	Correlation	Correlation	P-Value	Correlation
MONO	LYMPH (%)	-0.069	0.4015	-0.095	0.2459	0.006	0.9423
MONO	Abs. NEUT	0.502	<0.0001*	0.188	0.0211*	0.001	0.9910
MONO	NEUT (%)	-0.119	0.1479	-0.301	0.0002*	-0.018	0.8241
MONO	Abs. EOSIN	0.762	<0.0001*	0.426	<0.0001*	-0.007	0.9343
MONO	EOSIN (%)	0.368	<0.0001*	0.152	0.0632	0.015	0.8585
BASO	HCT (%)	0.052	0.5303	-0.182	0.0254*	-0.071	0.3875
BASO	HB (g/dL)	0.056	0.4971	-0.176	0.0308*	0.048	0.5578
BASO	RBC (x10^12/µL	-0.016	0.8464	-0.194	0.0171*	-0.017	0.8385
BASO	WBC (x10^3/µL)	0.331	<0.0001*	0.388	<0.0001*	0.089	0.2781
BASO	MCV (fl)	-0.153	0.0609	0.133	0.1036	0.096	0.2444
BASO	MCH (pg)	-0.076	0.3579	0.200	0.0139*	-0.001	0.9911
BASO	Abs. LYMPH	0.338	<.0001*	0.518	<.0001*	-0.070	0.3928
BASO	Abs. NEUT	0.214	0.0085*	0.386	<.0001*	0.031	0.7045
BASO	NEUT (%)	-0.156	0.0572	-0.051	0.5327	-0.098	0.2321
BASO	EOSIN	0.397	<.0001*	0.204	0.0121*	-0.076	0.3552
BASO	EOSIN (%)	0.429	<.0001*	-0.083	0.3111	0.079	0.3388
BASO	Abs. MONO	0.410	<.0001*	0.330	<.0001*	-0.027	0.7413
BASO	MONO (%)	0.127	0.1227	0.023	0.7842	0.190	0.0201*
PLT (x 10^3/µL)	HCT (%)	-0.128	0.1193	0.176	0.0311*	0.003	0.9683
PLT (x 10^3/µL)	HB (g/dL)	-0.138	0.0912	0.199	0.0149*	0.102	0.2155
PLT (x 10^3/µL)	MCV (fl)	-0.042	0.6140	-0.070	0.3971	-0.168	0.0394*
PLT (x 10^3/µL)	MCH (pg)	-0.176	0.0308*	-0.112	0.1708	0.004	0.9656

Significance Level:= p<0.05*

Table 2d. Pairwise correlations of hematological parameters by sickle cell anaemia subjects' state

Parameter	By parameter	Subjects state					
		Steady state (n=150)		Vaso-occlusive crisis (VOC) (n=150)		Control (n=150)	
		Correlation	P-Value	Correlation	Correlation	P-Value	Correlation
PLT (x 10^3/µL)	MCHC (g/dL)	0.219	0.0073*	0.067	0.4160	-0.033	0.6890
PLT (x 10^3/µL)	LYMPH (%)	-0.077	0.3463	-0.105	0.2022	-0.161	0.0492*
PLT (x 10^3/µL)	Abs. NEUT	0.098	0.2322	0.112	0.1719	-0.160	0.0509
PLT (x 10^3/µL)	NEUT (%)	0.070	0.3954	0.224	0.0058*	0.130	0.1122
PLT (x 10^3/µL)	Abs. EOSIN	0.092	0.2621	0.188	0.0214*	0.075	0.3588
PLT (x 10^3/µL)	Abs. MONO	0.132	0.1070	-0.089	0.2785	0.167	0.0412*
PLT (x 10^3/µL)	MONO (%)	0.011	0.8894	-0.168	0.0403*	-0.098	0.2324
MPV (fL)	HCT (%)	0.200	0.0141*	-0.034	0.6761	-0.126	0.1247
MPV (fL)	HB (g/dL)	0.181	0.0265*	-0.016	0.8416	-0.006	0.9425
MPV (fL)	MCV (fl)	0.120	0.1431	0.202	0.0131*	0.084	0.3063
MPV (fL)	NEUT (%)	-0.087	0.2916	0.167	0.0409*	-0.023	0.7753
MPV (fL)	Abs. MONO	-0.044	0.5940	-0.287	0.0004*	0.074	0.3656
MPV (fL)	MONO (%)	0.014	0.8632	-0.341	<.0001*	-0.071	0.3866
MPV (fL)	Abs. BASO	-0.099	0.2295	0.189	0.0206*	0.008	0.9234
MPV (fL)	BASO (%)	-0.121	0.1399	0.238	0.0033*	-0.010	0.9074
MPV (fL)	PLT (x 10^3/µL)	-0.090	0.2731	0.316	<.0001*	0.013	0.8705

Significance Level:= p<0.05*

There was occurrence of statistically significant (*p<.05*) trend of increase in lymphocytes, neutrophils, basophils and absolute eosinophils amongst the haematological parameters among subjects in the steady state and vaso-occlusive crisis. This result is corroborated by the finding of Zaini in 2019 [35], that slight increase in these parameters was detected.

This could be due to the impairment of the innate immunity which is the most well described immune dysfunction in individuals with SCA. Part of the dysfunction has been shown to include increased peripheral blood neutrophil count (granulocytosis), which often accounts for leukocytosis in SCA. However, the increased neutrophils are mostly dysfunctional due to impaired chemotaxis, migration and killing ability [36].

In keeping with the results of this study, a statistical significant positive correlation in the steady state (n=150) subjects, between haemoglobin and haematocrit (*r=.815*; *p<.001*), between Red blood cell and haematocrit (*r=.353; p<.001*), between mean cell haemoglobin and haemoglobin (*r=.241; p<.05*), between Mean cell haemoglobin concentration and mean cell volume (*r=.163; p<.05*), between absolute lymphocytes and haematocrit (*r=.164; p>.05*), and between lymphocytes and white blood cells (*r=.476; p<.001*).

This study is in support with Manas and colleagues in 2015 [37]. This could be due to the act of marginalizing ability of the immune cells to situate or initiate VOC. In addition, the significant correlation between MCH and haemoglobin in subjects with SCA has been associated with continuous haemolysis of red blood cells thereby reducing their survival to 10–20 days and splenic sequestration. Therefore, reduced haemoglobin concentration in subjects with SCA serves as a reflection of both degree of anaemia and degree of haemolysis [29].

Platelet was observed to correlate positively with neutrophil and negatively with lymphocyte as against the report of Chinawa and colleagues in 2013 [38]. This could be due to the ability of platelet to mediate leucocytes movement from bloodstream through the vessel wall to site of injury with neutrophil acting as phagocyte while lymphocytes act somewhat as antibody which is not well developed in the SCA subjects.

Mean platelet volume (MPV) correlate positively with monocytes, HCT and MCH, whereas MPV is inversely related to PLT count supported by Lippi and colleagues in 2015 [39] and Maina and colleagues in 2010 [40]. This is associated with bone marrow activation to produce platelet and rapidly release them into the circulation showing variation in size.

4. CONCLUSION

This study has shown that there are changes in haemtological parameters between the VOC and steady states of the SCA subjects as compared with the control subjects.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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