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ORIGINAL ARTICLE

Effect of Hydroxyurea Therapy on Markers of Inflammation and Clinical Variability of Sickle Cell Anaemia; A Prospective Study at a Missionary Hospital

Samuel Kwasi Appiah[1,2*], Charles Nkansah [1,2,] Gabriel Abbam[1], Felix Osei-Boakye[4], Samira Daud[1], Charles A. Derigubah[5], Opeabo-Sam Christian[3], Agyekum Abigail[3], Osei-Osarfo Emmanuel [3], Muniru Mohammed Tanko[3], Sibiri Ballu[6], Solomon Chemogo[6], Michael Asamoah Gyamfi[6], Eric Antwi Osei Kwadwo[6], Solomon Yeboah[6], Christopher Nkrumah[6], Bismark Nantomah[7], Victor U. Usanga[2], Boniface Nwofoke Ukwah[2], Ejike Felix Chukwurah[2]

[1]Department of Haematology, School of Allied Health Sciences, University for Development Studies, Tamale, Ghana.

[2]Department of Medical Laboratory Science, Faculty of Health Science and Technology, Ebonyi State University, Abakaliki, Nigeria.

[3]Department of Biomedical Laboratory Sciences, School of Allied Health Sciences, University for Development Studies, Tamale, Ghana.

[4]Department of Medical Laboratory Technology, Faculty of Applied Science and Technology, Sunyani Technical University, Sunyani, Ghana.

[5]Department of Medical Laboratory Technology, School of Applied Science and Arts, Bolgatanga Technical University, Sunyani, Ghana.

[6]Department of Medical laboratory & Orthopaedic, Methodist Hospital Wenchi, Bono Region, Ghana.

Abstract

Background: Immune mediators of inflammation are implicated in the pathophysiological processes and clinical variability of sickle cell anaemia (SCA). This study determined the effect of hydroxyurea therapy on markers of inflammation and clinical variability of sickle cell anaemia in Ghana.

Methods: This prospective case-control study was conducted at Methodist Hospital, Wenchi. Ninety participants (60 SCA patients and 30 apparently healthy controls (HbA)) aged 2-40 were recruited from March to July 2023. About 4 mL of blood was collected for complete blood count analysis using a Sysmex XN-550 haematology analyzer and serum C-reactive protein (CRP), interleukin (IL-6), and hepcidin using an enzyme-linked immunosorbent assay. Data were analyzed using SPSS version 27.

Results: Serum levels of IL-6 [4.7 (3.9-5.1) vs 3.0 (2.2-4.0), $p<0.001$], CRP [452.4±154.6 vs 267.5±84.2, $p<0.001$], NLR [1.4 (1.1-2.2) vs 0.9 (0.5-1.3), $p<0.001$], platelets [357.0 (248.5-445.5) vs 240.5 (180.5-264.5), $p<0.001$] and TWBC [10.3 (8.3- 12.6) vs 5.2 (4.3-5.9), $p<0.001$] were significantly elevated with reduction in levels of hepcidin [145.3 (109.2-185.2) vs 208.6 (150.6-246.4), $p<0.001$], HB [8.4±1.8 vs 12.8±1.3, $p<0.001$], RBC [2.6 (2.3-3.4) vs 4.5 (4.1-4.9), $p<0.001$] and HCT [25.6±5.4 vs 39.9±4.5, $p<0.001$] among SCA participants compared to the control group. However, the levels of the inflammatory markers showed significant reduction with improved haemogram in SCA participants treated with HU compared to the naïve group; [IL-6 ($p=0.014$), CRP ($p<0.001$), NLR ($p=0.009$), PLR ($p=0.003$), HB ($p<0001$), RBC ($p=0.001$), HCT ($p=0.031$), platelets ($p<0.001$), TWBC

[7]Department of Population and Reproductive Health, School of Public Health, University for Development Studies, Tamale, Ghana

***Corresponding Author:**
Samuel Kwasi Appiah
Email: appiahs30@yahoo.com

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(p<0.001)]. Levels of IL-6 (rho=0.503, *p<0.001*), CRP (rho=0.449, *p<0.001*), platelets (rho=0.554, *p<0.001*) and TWBC (rho=0.300, p=0.020) positively correlated with disease severity and VOCs incidence. NLR, PLR and hepcidin levels showed no significant correlation with disease severity score (p>0.05).

Conclusion: In summary, hydroxyurea therapy significantly improves red cell indices and reduces inflammatory biomarkers, which minimizes the severity of sickle cell anaemia complications.

Keywords: Cytokines, hydroxyurea, neutrophil-lymphocyte ratio, platelet-lymphocyte ratio; sickle cell anaemia.

INTRODUCTION

Sickle cell anaemia (SCA) is one of the most common genetic conditions globally, particularly among individuals of African descent with significant alteration in the morphology and qualitative function of erythrocytes resulting in a wide spectrum of clinical symptoms (1,2). Stress-induced polymerization of the mutant haemoglobin is a key component in the pathophysiology of sickle cell vaso-occlusive crisis (VOC). This causes an alteration in the biochemical and morphology of the red blood cells (RBC), resulting in a cascade of reactions involving the sickled cells, reticulocytes, leukocytes, platelets, and endothelial cells (3,4). The hallmark feature underlying the symptoms and problems associated with sickle cell anaemia, including pain, strokes, acute chest syndrome (ACS), priapism, and bone infarcts, is vascular-occlusive crises. Despite advances in supportive care, managing SCA remains challenging, prompting the exploration of novel therapeutic strategies to reduce its clinical burden (4,5).

One of the most effective therapeutic agents for managing sickle cell anaemia is hydroxyurea (HU), which inhibits the ribonucleotide reductase enzyme and increases the amount of foetal haemoglobin (HbF) in red blood cells. It also prevents sickle erythrocyte formation lowers leukocytes number which minimizes the frequency of VOCs and other complications (6,7).

Growing evidence describes sickle cell disease (SCD) as an inflammatory disorder. The acute-phase response by the innate immunity due to the SCD-induced endothelial cell activation and damage coupled with adhesion molecules provokes the release of pro-inflammatory cytokines such as interleukin (IL)-6, which promotes C-reactive protein (CRP, > 0.3mg/dL) and hepcidin synthesis (8,9). Hepcidin is a major regulator of iron metabolism. Its expression is upregulated in inflammation by IL-6 and other cytokines via the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway (10). Hepcidin and ferroportin form a complex and this complex is absorbed by these cells, where it causes ferroportin to degrade, inhibiting iron outflow and, as a result, reducing intestinal iron absorption and bioavailability (11,12).

Haematological markers serve as highly useful profiles for management of sickle cell anaemia and this is because the major clinical outcome of the disease may significantly increase or decrease some blood cell parameters. Some studies have reported reduced red blood cells indices and elevated white cell and platelets counts (13,14), whereas others have revealed an elevated levels of haematocrit and normal levels of leukocytes and platelets in SCD (15,16). Platelet-lymphocyte ratio

(PLR) and neutrophil-lymphocyte ratio (NLR) have been significant prognostic implications in several inflammatory conditions including malignancies, renal, gastrointestinal, cardiovascular disorders and SCD. The leucocyte ratios can be predictive of patients' reactions to inflammatory injury as consequence of reduced lymphocyte population caused by apoptosis, whereas neutrophils proliferate in response to stress (9,17,18). Despite the numerous studies targeting NLR, PLR, CRP and IL-6 as biomarkers inflammation in other conditions, limited information is known on how these markers influence the clinical variability of SCA. Also, while the efficacy of HU in reducing the frequency of vaso-occlusive crises and improving haematological parameters in SCA has been reported, its impact on markers of inflammation and the clinical variability of the disease warrants further investigation.

MATERIALS AND METHODS

Study site/design

This prospective case-control study was conducted between March 15th to July 20th, 2023 at the Methodist Hospital, Wenchi, a Christian faith-based hospital in the Bono Region of Ghana. The 250-bed hospital acts as a referral point for 20 public and private healthcare facilities in the Wenchi municipality and sections of the Savannah region. The hospital provides specialized surgical services in orthopaedics, urology, and general surgery. The digital address for the hospital is BW-0005-0306. According to the 2021 population and housing census, the municipality's population is 124,758, with 60,960 males and 63,798 females, with farmers constituting the greater portion (19).

The Institutional Review Board of University for Development Studies, Ghana approved the study (UDS/RB/006/23). Permission was sought from the management of Methodist Hospital, Wenchi. The study's purpose and procedures were described to the participants, and either their written or verbal consent or that of their legal guardian if they were under the age of 18years was acquired. Participants below the age of 2 years, with haemoglobin variants other than HbS, comorbidities such as diabetes mellitus, hypertension, human immunodeficiency virus, hepatitis, malaria, other haematological abnormalities, ppregnant women and lactating mothers were excluded from this study. Socio-demographic and clinical information were obtained from the clinical records. Severity scores were calculated based on the patients' clinical data as proposed earlier by Hedo et al. (20). The total score was classified as mild SCA (Score <3), moderate SCA (Score 3 and ≤ 7) and severe SCA (Score > 7) (20).

Laboratory Investigations

About 4 mL of venous blood samples from each patient was aseptically taken and divided into two tubes: 2 mL into EDTA, and 2 mL into gel tube. The EDTA sample was used for complete blood count estimation, sickling test and Hb electrophoresis. The gel tube sample was allowed to clot and spun at 3500 rpm for five minutes. The resulting serum was transferred into Eppendorf tubes and stored at -20°C for hepcidin, CRP and IL6 estimation using enzyme-linked immunosorbent assay (ELISA).

Full Blood Count Measurement

Complete blood count for each participant was estimated using an automated haematology analyzer (Sysmex XN-550, Japan) following the manufacturer's protocol. The analyzer works on the principles of impedance for the measurement of red blood cells and platelets, flow cytometry for the assessment of white blood cells and differentials, and colorimetry for the estimation of haemoglobin. Neutrophil-lymphocyte ratio and platelet-lymphocyte ratio were calculated by dividing the absolute neutrophil count by the absolute lymphocyte count and the absolute platelet count by the absolute lymphocyte count, respectively.

Biochemical determination

Serum CRP, IL-6 and hepcidin levels were assayed by the sandwich ELISA method using commercially prepared ELISA kits (Biobase, China). The ELISA procedures were performed according to the manufacturer's instructions. The ELISA plates were washed and read using semi-automated microplate washer and reader (Poweam, China).

Statistical Analysis

Statistical Package for Social Science (SPSS) version 27.0 was used for the statistical analysis. Tests for normality were done with Kolmogorov-Smirnov test. Non-parametric variables were reported as median and interquartile, whereas all parametric variables were presented as mean standard deviation. Bivariate parametric and non-parametric data were compared using Student' T-test and Mann-Whitney U-Test, respectively. Pearson and Spearman correlation tests were used to test for correlation between two continuous parametric and non-parametric data, respectively. A $p< 0.05$ was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics of the Study Participants

Of the 90 participants recruited into the study, 60 (66.7%) were sickle cell anaemia patients and 30 (33.3%) were apparently healthy individuals. Forty-one (45.6%) were males and 49 (54.4%) were females, with median age of 16.0 (2.0-34.0) years. More than half of the cases (32/ 53.3%) were on hydroxyurea therapy. Most (42/70%) of the cases visited the hospital between 1-3 times per year whiles (10/16.7%) visited the hospital more than 3 times per year. Majority (38/63.3%) of the patients experienced between 1 to 3 times vaso-occlusive crises per year, 16 (26.7) had crises more than 3 times and (6/10.0%) experienced no crises per annum. About 42% of the cases transfusion between 1 to 2 times per annum whereas 30% were transfused more than 3 time per year. In terms of severity score, 34 (56.7%) of the subjects were mild, 22 (36.7%) were moderate and 4 (6.6%) were severe (Table 1).

Table 1: Demographic and Clinical Characteristics of the Study Participants

Variable	Category	Frequency (%)
Age (years)	16.0 (2.0-34.0)	
Sex	Males	41 (45.6)
	Females	49 (54.4)
Hydroxyurea usage	Yes	32 (53.3)
	NO	28 (46.7)
Frequency of hospital visit per year	None	8 (13.3)
	1-3	42 (70)
	>3	10 (16.7)
Number of blood transfusions per year	0	17 (28.3)
	1-2	25 (41.7)
	≥ 3	18 (30.0)
Vaso-occlusive crises per year	None	6 (10)
	1-3	38 (63.3)
	>3	16 (26.7)
Severity score category	Mild	34 (56.7)
	Moderate	22 (36.7)
	Severe	4 (6.6)
Severity Score average	3.0 (2.0-4.8)	

Categorical data is presented in frequencies with corresponding percentages in parentheses. Age was presented as median (25^{th}-75^{th} percentiles).

Blood Cell Parameters of the Study Participants Stratified by SCA Cases and Controls

the Complete blood count parameters; RBC ($p<0.001$), Hb ($p<0.001$) and HCT ($p<0.001$ were significantly decreased among SCA participants compared to the controls whereas MCV ($p<0.001$), MCH ($p=0.019$), MCHC ($p=0.002$], RDW-CV ($p<0.001$), TWBC ($p<0.001$] and Platelet ($p<0.001$) were significantly higher in cases than the control group (Table 2).

Table 2: Blood Cell Parameters of Study Participants Stratified by SCA Cases and Controls

Blood Cell Parameters	Participants		*p*-value
	SCA Cases (N= 60)	Controls (N= 30)	
RBC x10^3/µl	2.6 (2.3-3.4)	4.5 (4.1-4.9)	**<0.001**
Hb (g/dL)	8.4±1.8	12.8±1.3	**<0.001**
HCT%	25.6±5.4	39.9±4.5	**<0.001**
MCV (fL)	89.4±13.4	80.3±6.1	**<0.001**
MCH (pg)	30.1±4.3	28.3±2.7	**0.019**
MCHC (g/dL)	34.6 (33.4-36.0)	32.8 (30.7-34.7)	**0.002**
RDW-CV%	18.2±3.7	8.3±0.7	**<0.001**
TWBC ×/µL	10.3 (8.3- 12.6)	5.2 (4.3-5.9)	**<0.001**
PLT. x10^3/µl	357.0 (248.5-445.5)	240.5 (180.5-264.5)	**<0.001**

N = Number of participants, g/dL=Gram per deciliter, %= percentage, µL= microliter, fL= Femtoliter, pg= Picogram, SCA= Sickle cell anaemia, RBC = Absolute red blood cell count, Hb = Haemoglobin concentration, HCT = Haematocrit, MCV = Mean cell volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration, RDW-CV = Red blood cell distribution width-coefficient of variation, TWBC = Total white blood cell count, PLT = Platelet count. Parametric data presented as mean ± standard deviation was compared using Student T test, and non-parametric data presented as median (25^{th}-75^{th}) were compared using Mann- Whitney U-test. P< 0.05 was deemed statistically significant.

Inflammatory Biomarkers of Study Participants Stratified by SCA Cases and Controls The levels of serum IL-6 (p<0.001), CRP (p<0.001), and NLR (p<0.001) were significantly higher in SCA cases than the controls, but serum hepcidin levels showed significant reduction in cases compared to the controls (p<0.001) respectively (Table 3).

Table 3: Inflammatory Biomarkers Stratified by SCA Cases and Controls

Inflammatory Biomarkers	Participants		*p*-value
	SCA Cases (N= 60)	Controls (N= 30)	
IL-6 (pg/mL)	4.7 (3.9-5.1)	3.0 (2.2-4.0)	**<0.001**
CRP (µg/L)	452.4±154.6	267.5±84.2	**<0.001**
Hepcidin (µg/L)	145.3 (109.2-185.2)	208.6 (150.6-246.4)	**<0.001**
NLR	1.4 (1.1-2.2)	0.9 (0.5-1.3)	**<0.001**
PLR	100.4 (68.4-154.2)	95.9 (67.4-144.0)	0.751

N = Number of participants, µg/L = microgram per litre, pg/mL = Picogram per milliliter SCA= Sickle cell anaemia, IL-6= Interleukin-6, CRP= C-reactive protein, NLR=Neutrophil-Lymphocyte ratio, PLR=Platelet-Lymphocyte ratio; Parametric data presented as mean ± standard deviation was compared using Student T test, and non-parametric data presented as median (25^{th}-75^{th}) were compared using Mann- Whitney U-test. P< 0.05 was deemed statistically significant.

Effect of Hydroxyurea Therapy on Haemato-immunological Biomarkers

The red cell indices; RBC (p=0.001), Hb (p<0.001) and HCT (p=0.031) improved significantly in SCA patients on HU therapy compared to the HU-naïve group whereas TWBC (p<0.001) and Platelet (p<0.001) counts significantly decreased in SCA patients on HU therapy. On the other hand, serum levels of inflammatory biomarkers [IL-6 (p=0.014) and CRP (p<0.001)] and haematological ratios [NLR (p=0.009) and PLR (p=0.003)] significantly decreased in SCA patients on HU therapy than their counterparts without HU. However, hepcidin levels did not differ between those on HU and those without HU therapy (p=0.684) (Table 4).

Table 4: Haemato-immunological Biomarkers of Study Participants Stratified by Hydroxyurea Usage

Haemato-immunological Parameters	Participants		*p*-value
	HU Therapy Cases (N= 32)	HU Naïve Cases (N= 28)	
RBC x10^3/µl	3.0 (2.5-4.1)	2.3 (2.0-2.7)	**0.001**
Hb (g/dL)	9.2±1.5	7.5±1.5	**<0.001**
HCT%	27.0±4.8	24.0± 5.6	**0.031**
TWBC x10^3/µl	9.1 (7.6- 10.9)	12.0 (9.6-14.3)	**<0.001**
PLT. x10^3/µl	262.0 (208.3-360.0)	441.0 (357.0-543.5)	**<0.001**
NLR	1.1 (0.8-1.1)	1.2 (1.1-1.7)	**0.009**
PLR	74.2 (56.9-114.3)	130.7 (92.2-180.4)	**0.003**
IL-6 (pg/mL)	4.4 (3.8-5.1)	5.0 (4.5-7.0)	**0.014**
CRP (µg/L)	345.0 (244.9-456.0)	543.0 (452.7-672.0)	**<0.001**
Hepcidin (µg/L)	137.43 (108.8-184.3)	157.8 (110.7-190.2)	0.684

N = Number of participants, g/dL=Gram per deciliter, %= percentage, µL= microliter, pg/mL = Picrogram per milliliter, RBC = Absolute red blood cell count, Hb = Haemoglobin concentration, HCT = Haematocrit, TWBC = Total white blood cell count, PLT = Platelet count, IL-6= Interleukin-6, CRP= C-reactive protein, NLR=Neutrophil-Lymphocyte ratio, PLR=Platelet-Lymphocyte ratio; Parametric data presented as mean ± standard deviation was compared using Student T test, and non-parametric data presented as median (25^{th}-75^{th}) were compared using Mann- Whitney U-test. P< 0.05 was deemed statistically significant.

Relationship between Inflammatory Biomarkers and Clinical Variability

A significant moderate positive correlation was observed between disease severity score and IL-6 (rho=0.503, p<0.001), CRP (r=0.449, p<0.001) and Platelets (rho=0.554, p<0.001) respectively, but weak positive correlation was observed with TWBC (rho=0.300, p=0.002). also, VOC incidence positively corelated with and IL-6 (rho=0.330, p=0.010), CRP (r=0.485, p<0.001) TWBC (r=0.421, p<0.001) and Platelets (rho=0.487, p<0.001). However, correlation between disease severity score and VOC with NLR, PLR and hepcidin were not statistically significant (Table 5).

Table 5: Correlation of Inflammatory Parameters with Clinical Variability

Immunological Biomarkers	Disease Severity Score		VOC	
	Rho	*P*-value	Rho	*P*-value
IL-6 (pg/mL)	0.503**	**<0.001**	0.330*,	**0.010**
CRP (µg/L)	0.449**	**<0.001**	0.485**	**<0.001**
Hepcidin (µg/L)	-121	0.356	0.056	0.668
NLR	0.021	0.874	0.192	0.142
PLR	0.157	0.231	0.204	0.118
TWBC x10^3/µl	0.300*	**0.020**	0.421**	**<0.001**
PLT x10^3/µl	0.554**	**<0.001**	0.487**	**<0.001**

rho= Correlation coefficient, uL= microliter, VOC=Vaso-occlusive crisis, SCA= Sickle cell anaemia, IL-6= Interleukin-6, CRP= C-reactive protein. Spearman correlation test was used to determine the correlation between disease severity, and WBC, IL-6, NLR, platelets, hepcidin and PLR, whiles Pearson correlation was employed for CRP analysis. P value was significant at 0.05 * and 0.01 or less **.

DISCUSSION

Inflammation is the hallmark of the clinical symptoms and complications associated with SCA.21 Growing evidence implicate haematological ratios such as NLR, PLR and immunological markers (IL-6, CRP and hepcidin) to play pivotal role in the pathogenesis of SCA clinical symptoms and complications (18). Evaluating the changes of these biomarkers may have a potential value in monitoring the progress and resolution of SCA crisis. There is paucity of data on the effect of HU on haematological ratios and the immunological cytokine among Ghanaian SCA patients, and therefore the need to determine the effect of HU therapy on the inflammatory markers and clinical variability of SCA patients in Ghana.

In this study, participants with SCA had lower red cell parameters (Hb, RBC and HCT) and elevated MCV, MCH, MCHC and RDW-CV compared to the control group which is consistent with earlier findings in Ghana (14) Nigeria (23,24), Congo (25) and Central Iraq (26). The decreased levels of Hb, RBC, HCT could be attributed to chronic haemolysis, shortened red cell survival, inflammation and a diminished erythropoietin response

related to SCA (27,28) The high MCV, MCH, MCHC and RDW-CV observed among participants with SCA in this study is consistent with poikilocytosis, macrocytosis and reticulocytosis as a consequence of bone marrow compensative mechanism in response to the anaemia (4)

Leukocytes and thrombocytes have both been reported to increase during chronic inflammatory conditions contributing to the pathophysiology of the SCA severity and morbidity (6,22). This present study reported significantly elevated white blood cell and platelet counts in SCA patients than the control group. The significant leukocytosis observed in this study could be explained by the persistent, subclinical inflammation that results in cytokine release that invigorates bone marrow leucocyte production or the functional hyposplenia (25). The high platelet counts seen among patients with SCA could be reactive in response to the inflammation and hyposplenism associated with the disease pathophysiology (29,30). Another factor contributing to thrombocytosis in SCA may be the negative feedback effect of anaemia on erythropoietin production, given the structural similarities between erythropoietin and thrombopoietin, thrombocytosis can accompany anaemia of chronic diseases as well as other kinds of anaemia (31)

In the current study, serum levels of IL-6 were significantly higher among SCA participants compared to the control group, and this is similar to findings from earlier studies (13,32) This finding could be attributed to the chronic haemolysis, vaso-occlusive events, and ischemia-reperfusion injury which precipitate inflammatory response leading to increased production of IL-6, a pro-inflammatory cytokine (32).

Similarly, the markedly higher levels of CRP observed among SCA participants suggests heightened systemic inflammation as part of a non-specific acute phase response. This finding can be attributed to the fact that chronic haemolysis and inflammation in SCA contribute to increased CRP production by the liver as an acute-phase reactant (32). This is in line with earlier findings reported in Nigeria (33,34).

Interestingly, serum hepcidin levels were significantly reduced in SCA patients compared to the control group, and this is in line with a study conducted in Brazil (13). This finding can be attributed to the fact that, in conditions like SCA, where chronic anaemia coexists with inflammation, there could be a paradoxical suppression of hepcidin as a result of the overriding effect of erythropoietic stress. This suppression allows for increased iron absorption to support erythropoiesis despite the stimulatory effect of IL-6 inflammatory cytokine on hepatic hepcidin synthesis (35).

Neutrophil-lymphocyte ratio and platelets-lymphocyte ratio indices are recent emerging markers known to play significant role in inflammatory process in chronic diseases like SCA. Although there have been inconsistent reports regarding changes in the number of immune inflammatory mediators in SCA patients (36,38). This present study reported significantly higher NLR among SCA cases than in control group which corroborates earlier studies (10,19). This finding could be attributed to critical role leukocytes play in the pathophysiology of sickle cell crisis. The transition of SCA pathology from steady state to VOC is promoted by elevated and activated leukocytes. By secreting pro-inflammatory mediators coupled with expression of related adhesion molecules facilitate the adherence of the leukocytes to the endothelium worsening the VOC and pains associated with SCA. Also, the activation of immune responses against damaged red blood cells and the release of inflammatory mediators in SCA may contribute to alterations in leukocyte ratios (24). Our finding contradicts the observation reported by an earlier study (35). The variation in the findings may be attributed to

demographics and differences of the study subjects. Whiles this study recruited SCA patients without any comorbidity, the study by Emokpae and co (35) included SCA patients with renal insufficiency. However, PLR did not show a significant difference between SCA cases and controls. The lack of significant difference in PLR may indicate that PLR does not vary substantially in SCA cases compared to controls in the context of inflammation.

Again, hydroxyurea was found to improve significantly the red cell indices and reduces the levels of inflammatory biomarkers (WBC, Platelet, NLR, PLR, IL-6 and CRP) among SCA patients on HU therapy compared to the HU-naïve participants. This finding is in consonance with previous studies conducted in USA (40) and India (39). The plausible reason could be attributed to the fact that hydroxyurea acts by increasing the production of fetal haemoglobin, which has a higher affinity for oxygen than HbS. The higher levels of HbF result in improved RBC flexibility and decreased sickling. This, in turn, leads to an increase in the overall number of functional red blood cells, reflected in elevated RBC count, Hb concentration, and HCT%. Also, hydroxyurea has been shown to downregulate pro-inflammatory cytokines and acute phase proteins including IL-6, and CRP (39,40).

Inflammatory biomarkers (IL-6, CRP, TWBC and platelets) showed a strong positive correlation with increased disease severity which is consistent with findings from earlier study. (33,40,41). This may be due to the complicated interplay between sickled erythrocytes, endothelial cells, leukocytes, platelets, and activation of pro-inflammatory cytokines underlying the pathophysiology of vaso-occlusive crisis, which is the hallmark of SCA complications (39). This finding is in contrast with a study conducted in Nigeria that reported negative correlation between CRP and disease severity (34). This contrasting finding may be due to the variations in the study participants and geographical locations. Whiles the current study was conducted among steady state SCA patients' 2-40 years in Ghana, the study by Okacha and co. recruited participants in both steady state and in vaso-occlusive crisis in Nigeria. The study found no correlation between disease severity; and NLR, PLR and hepcidin which agrees with previous studies (18,42). The reason for this finding could possibly be attributed to the cytotoxic effect of HU on the immune cells. The study was done on only steady state clients and did assess other acute-phase reactants.

CONCLUSION

Interleukin-6, CRP, NLR platelets and TWBC immunological biomarkers of inflammation were significantly higher with reduction in HB, RBC and HCT among SCA participants compared to the control group. Hydroxyurea was found to improve red cells indices and reduces the inflammatory biomarkers which minimizes SCA complications. These inflammatory biomarkers may provide additional diagnostic and prognostic information regarding the management of SCA and its clinical complications. Further studies are required on a broader scale to determine if these immunological markers can be used to predict the occurrence of crisis in SCA patients.

AUTHORS' CONTRIBUTIONS

All authors contributed significantly to see the success of this work. Authors also approved the final version of the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conceptualization: S.K. Appiah.

Study design: S.K. Appiah, C. Nkansah, G.

Abbam C. Opeabo-Sam, A. Agyekum, S. Ballu

Data curation: S.K. Appiah, F. Osei-Boakye, C. Nkrumah, Solomon Chemogo

Laboratory investigations: S. K. Appiah, E. A. K. Osei, S.Yeboah, S. Daud, C.A. Derigubah

Statistical analysis: S. K. Appiah, F. Osei-Boakye, C. Nkansah, M. A. Gyamfi

Resources: S. Chemogo, G. Abbam, C. Opeabo-Sam, A. Agyekum, B. Nantomah

Supervision: S.K. Appiah, V.U. Usanga, B.N. Ukwah, E.F. Chukwurah, S. Ballu, M. A. Gyamfi, B. Nantomah

Writing - original draft: S. K. Appiah, C. Opeabo-Sam, A. Agyekum, C. Nkansah

Writing – review & editing: S. K. Appiah, S. Ballu, F. Osei-Boakye V. U. Usanga, B.N. Ukwah, E. F. Chukwurah

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Data Availability: All relevant data are within the article. The original data used to support the findings of this study are available from the corresponding author upon reasonable request.

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