ORIGINAL RESEARCH ARTICLE

Redefining the Reference Interval and Cut-Off Values of the Hematological and Biochemical Parameters and Deriving a Sensitive Predictive Marker for Crisis Events in Sickle Cell Disease

Suprava Patel[1] · Diksha Chandrakar[1] · Preetam N. Wasnik[2] · Eli Mohapatra[1] ·
Rachita Nanda[1] · Seema Shah[1] · Ashish Gupta[3]

Abstract The heterogeneity in clinical presentations in sickle cell disease (SCD) alters between crisis and steady state phases. Considering the pathophysiology, it is crucial to establish a disease-specific reference interval for hematological and biochemical parameters and identify the sensitive predictive markers for crisis. The case–control study included fifty-four healthy control, forty SCD cases in crisis state, and forty-six steady state cases. Blood samples were evaluated for complete blood count (CBC) and biochemical parameters. Red cell indices were calculated. 2.5th and 97.5th percentile values were delineated in the steady state cases as new reference interval. The new cut-off values were evaluated for sensitivity and specificity. The steady state cases reported a significant decrease in urea, sodium, Hb, Hct, and RBC count compared to the control group, whereas potassium, and red cell indices were higher. Compared to the steady state, the crisis state depicted a significant increase in urea, liver enzymes, leucocytes, inflammatory indices, and all red cell indices, whereas sodium, albumin, iron, Hb, Hct, and LMR were decreased. The revised 95 percentile ranges were sodium-132.35–143.65 mmol/L, potassium-3.75–6.09 mmol/L, albumin-2.9–5.0 g/dL, Hb-7.51–12.17 g/dL, RBC count-2.2–5.49×10^6/μL, and Hct-22.54–36.1%. Red cell indices like Ricerca, Green and King, and Huber–Heklotz could be good screening tools for crisis events with more than 70% sensitivity. Thus, the laboratory must derive a new reference interval and cut-off values at the steady state in the SCD population it is catering samples rather than comparing it with normal healthy individuals.

Keywords CBC profile · Serum markers · Crisis state · Steady state · Percentile · ROC analysis

✉ Suprava Patel
dr_suprava@yahoo.co.in

Diksha Chandrakar
dikshachandrakar18@gmail.com

Preetam N. Wasnik
pnwasnik@aiimsraipur.edu.in

Eli Mohapatra
dr.e.mohapatra@aiimsraipur.edu.in

Rachita Nanda
dr.rachitananda@aiimsraipur.edu.in

Seema Shah
sshah@aiimsraipur.edu.in

Ashish Gupta
ashish_bmc@yahoo.co.in

1 Department of Biochemistry, All India Institute of Medical Sciences, Raipur, Chhattisgarh, India

2 Department of Medicine, All India Institute of Medical Sciences, Raipur, Chhattisgarh, India

3 Department of Pathology, All India Institute of Medical Sciences, Raipur, Chhattisgarh, India

Introduction

A point mutation (GAG to GTG) in the *hemoglobin beta gene* (*HBB*) on chromosome 11p15.5 results in the substitution of amino acid glutamic acid (hydrophilic) with valine (hydrophobic) in the sixth position of the beta-globin chain results in the production of hemoglobin S (HbS) variant instead of the normal adult hemoglobin (HbA) [1]. Individuals heterozygous for this mutated gene are referred to as sickle cell trait (SCT), and who are homozygous are referred

to as sickle cell disease (SCD). SCD is the most common inherited hemoglobinopathy in Central India, with a prevalence of 9.3% heterozygous form and 0.21% homozygous SCD form [2, 3]. Study findings have reflected that, in the absence of provision for early diagnosis through newborn screening or mass screening due to fund constraints in developing countries like India, individuals with SCD die very young due to late diagnosis. Individuals with SCD have reported a broad spectrum of clinical heterogeneity in their presentations. SCT cases usually remain asymptomatic and have normal blood parameters comparable to their healthy counterparts. However, SCD cases experience alternating episodes of crisis phases and steady state [4].

SCD, primarily a disorder of red blood cells (RBCs), causes alterations in their structural integrity, rheological properties, and biological function. The sickle-shaped RBCs lose their flexibility to pass through small capillaries and lead to occlusion of the microvascular network. Repeated episodes of clogging and dislodgement eventually end up with hemolysis and initiate the crisis event [5]. The pathophysiology of this vaso-occlusive crisis (VOC) affects the RBCs and the other cellular elements in the blood, such as leucocytes and platelets. Repeated perfusion-reperfusion events often result in organ damage and changes in various biochemical parameters during the crisis event [6]. Identifying the biomarkers that could be sensitive enough to predict the shift from a steady state to a crisis state is essential. Early evaluation of these markers might be beneficial in reducing the episodes of severe hemolytic and painful crises, frequency of hospitalization, requirement for transfusions, and other severe complications associated with SCD. It is also essential in countries with low resource settings that these parameters should be easily estimated at nearby peripheral health care centers and should be affordable for the patients. Identifying a sensitive marker would be of utmost benefit because it could be a good screening tool for monitoring the patient's progress toward crisis.

Individuals with SCD are in their steady state; though it seems normal, the underlying pathophysiological changes continue at a subclinical level. Because of the ongoing process, the laboratory reference interval of the various hematological and biochemical parameters in steady state SCD cases are expected to differ from those in a healthy individual [7]. These individuals manifest the symptom only when the changes become overwhelming. Therefore, it is of utmost importance that the reporting laboratories establish a disease-specific reference interval for SCD cases at their steady state in their population. An established reference interval is critical for clinicians in decision-making. The new revised reference values would help the clinician for an early assessment of the disease course towards crisis state by comparing the changes in the steady state reference interval of blood parameters.

Not many laboratories have established a reference interval for SCD cases in their population; thus, cut-off values and sensitive screening markers are yet to be defined. Looking into the current practices of reporting the values against the biological reference interval of a healthy individual, we tried to observe the overall hematological and biochemical changes in steady state and crisis state SCD cases in our population. The aim was to evaluate the disease-specific reference interval at steady state for these parameters and identify the sensitive screening markers for early assessment of the progression of the clinical course from steady state to crisis state.

Materials and Methods

The case–control study included one hundred forty adults, of which eighty-six were SCD cases, and fifty-four were healthy control group. The institute's ethics committee approved the study protocol, and written informed consent was taken from all participants. Individuals who volunteered for blood donation in our institute and found to be apparently healthy were recruited and enrolled as the control group. Cases already confirmed for homozygous (HbSS) were included in the study. Individuals with any noticeable or known acute or chronic disorders in last 6 months (except for SCD in cases), received blood transfusion in last 12 weeks and pregnant females were excluded from the study. The cases were categorized into crisis state (n = 40) and steady state (n = 46) as per clinical presentation, present or absent. SCD cases with no obvious clinical symptoms and attending the outpatient department (OPD) for routine follow-up were considered steady state cases. Others who presented with clinical features as tabulated in Table$_{suppl}$-1, were categorized under crisis state. Cases with a history of blood transfusion within the last four months were not enrolled for the study. The clinical details were recorded, and as per the clinical presentations, all cases were assigned a severity score as per Adegoke and Kuti scoring system. Blood samples were collected from each participant for analysis of serum renal function tests (RFT), liver function tests (LFT), iron, HbF, and complete blood count (CBC). The serum samples were analyzed for all the quantitative variables in AU 5811 fully automated autoanalyzer from Beckman Coulter, and complete blood count (CBC) parameters were analyzed in an Automated Hematology analyzer from Sysmex. Blood HbF levels were analyzed by high-performance liquid chromatography (HPLC) in the D-10 Hemoglobin testing system from Biorad. The biological reference interval are as per the manufacturer's instructions in the kit inserts for all these parameters are provided in Table$_{suppl}$-2.

The inflammatory and red cell indices were calculated for all participants. The following equations were applied for the calculation [8, 9]:

Inflammatory indices:

- Lymphocyte-monocyte ratio (LMR) = Lymphocyte/Monocyte
- Neutrophil-monocyte ratio (NMR) = Neutrophil/Monocyte
- Neutrophil–lymphocyte ratio (NLR) = Neutrophil/Lymphocyte
- Systemic immune-inflammatory index (SII) = Platelet x NLR

Red cell indices:

- Mentzer index (MI) = mean corpuscular volume/red blood cell count (MCV/RBC)
- Red cell distribution width (RDW) index (RDWI) = MCV × RDW/RBC
- Green and King index (GKI) = $MCV^2 \times RDW/(Hb \times 100)$ [10]
- Ehsani index (EI) = MCV–10 × RBC [11]
- England and Fraser index (EF) = MCV–(5 × Hb)–RBC–3.4
- Ricerca index (RI) = RDW/RBC
- Srivastava index (SI) = Mean corpuscular hemoglobin (MCH)/RBC
- Shine and Lal index (SLI) = MCV × MCV × MCH/100 [12]
- Huber–Heklotz index (HHI) = MCH × RDW × 0.1/RBC + RDW [13]
- Sirdah index (SirI) = MCV–RBC–(3 × Hb) [14]

Statistical Analysis

The statistical analyses were performed by IBM@SPSS version 26. The quantitative parameters were depicted as mean with standard deviation (SD). The values were compared among the three study groups using analysis of variance (ANOVA) with a posthoc test. The 2.5th and 97.5th percentile values were calculated for all the parameters in the three study groups. The 2.5–97.5th percentile range in the steady state group was considered the baseline or new reference interval for comparison for the crisis state.

Receiver operating characteristic (ROC) curve analysis in cases was performed for all parameters for crisis event. Parameters showing an area under curve (AUC) of more than 0.6 were further considered to derive the cut-off value. Two methods determined cut-off values. In the first method, the mean values of these parameters (AUC > 0.6) in the steady state individuals were considered the new cut-off value. The second method was deriving an optimum cut-off value for a crisis event derived from the ROC curve. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for crisis events were calculated for both the cut-off values. The cut-off value with a greater AUC and higher sensitivity percentage was considered a good screening tool for predicting crisis events.

Result

The study population comprised eighty-six homozygous (HbSS) SCD cases and fifty-four healthy individuals. No significant differences were observed for age group and the gender distribution among the two study groups. The mean (SD) age was 21.67 (5.23) in cases and 22.9 (2.56) in the control group (*p* = 0.125). About 67.1% (n = 94) were males, and 32.9% (n = 46) were females (*p* = 0.17) in the total study population. In the cases, 62.8% were males and 37.2% were females, whereas the percentages were respectively 74.1% and 25.9% in the control group. The male:female ratio was 1.69 (54/32) in cases and 2.86 (40/14) in the control group. In the SCD crisis group, 70% (n = 28) of subjects were males, whereas the female percentage was 30% (n = 12). The odds for crisis events in SCD cases was 1.79 (95%CI 0.735–4.38, *p* = 0.19) in males as compared to females.

The mean (SD) comparison of the serum markers and the CBC profile is delineated in Table 1**.** Mean serum urea was significantly higher in crisis state (20.53 mg/dL) than in steady state cases (15.63 mg/dL; *p* = 0.031). Compared to the control group, the level was significantly lower in the steady state cases (*p* = 0.01). Serum creatinine was greatly reduced in the cases than the control group. Similarly, the cases depicted grossly reduced serum sodium than the control group (*p* < 0.001). The crisis group patients revealed significant hyponatremia (136.35 mmol/L) compared to the steady state group (*p* = 0.025). No significant difference in serum potassium was observed between the crisis and steady state cases. However, the steady state group reported elevated serum potassium (4.36 mmol/L) compared to the control group (*p* = 0.004). The crisis state cases depicted significantly elevated LFT parameters such as serum direct bilirubin (D. Bil), aspartate transaminase (AST), and alkaline phosphatase (ALP) levels than the steady state and control individuals. On the contrary, total protein, albumin, albumin-globulin ratio (AGR), and iron levels in the serum were lower. The serum gamma-glutamyl transferase (GGT) was higher in the crisis group compared to the healthy control group (*p* = 0.003).

In the CBC panel, mean blood hemoglobin (Hb), hematocrit (Hct), and red blood cell (RBC) count were grossly reduced in both cases than the control group (*p* < 0.001), whereas red cell distribution width (RDW) and fetal hemoglobin (HbF) values were elevated.

Table 1 Comparison of mean (SD) of the quantitative variables among the study groups (N = 140)

Parameters (units)	Crisis State[a] (n = 40)	Steady State[b] (n = 46)	Control Group[c] (n = 54)	p-values		
				a vs b	a vs c	b vs c
Urea (mg/dL)	20.53 (14.3)	15.63 (5.2)	20.89 (5.4)	0.031*	0.98	0.01*
Creatinine (mg/dL)	0.75 (0.5)	0.68 (0.1)	0.89 (0.1)	0.47	0.038*	0.001*
Uric acid (mg/dL)	5.37 (2.9)	5.09 (1.2)	5.6 (1.4)	0.77	0.84	0.84
Sodium (mmol/L)	136.35 (4.0)	137.98 (2.4)	140.56 (2.0)	0.025*	< 0.001*	< 0.001*
Potassium (mmol/L)	4.36 (0.6)	4.58 (0.5)	4.24 (0.4)	0.11	0.53	0.004*
Chloride (mmol/L)	100.95 (5.9)	102.57 (2.6)	103.44 (2.0)	0.12	0.005*	0.47
T. Bil (mg/dL)	3.24 (3.1)	2.51 (1.1)	0.89 (0.4)	0.16	< 0.001*	< 0.001*
D. Bil (mg/dL)	1.01 (1.6)	0.53 (0.3)	0.18 (0.1)	0.038*	< 0.001*	0.13
I. Bil (mg/dL)	2.2 (1.7)	1.98 (0.9)	0.69 (0.4)	0.61	< 0.001*	< 0.001*
AST (U/L)	54.25 (33.5)	35.87 (13.9)	27.89 (14.3)	< 0.001*	< 0.001*	0.16
ALT (U/L)	27.93 (17.3)	22.33 (15.6)	25.63 (16.7)	0.26	0.78	0.58
ALP (U/L)	136.73 (84.5)	97.43 (37.8)	90.96 (17.4)	0.002*	< 0.001*	0.8
GGT (U/L)	47.73 (46.7)	33.7 (49.7)	20.74 (7.9)	0.21	0.003*	0.21
Total protein (g/dL)	7.47 (0.9)	8.08 (0.5)	7.89 (0.5)	< 0.001*	0.007*	0.29
Albumin (g/dL)	3.81 (0.6)	4.47 (0.4)	4.59 (0.3)	< 0.001*	< 0.001*	0.4
Globulin	3.66 (0.9)	3.61 (0.6)	3.3 (0.3)	0.94	0.032*	0.06
AG ratio	1.11 (0.3)	1.29 (0.3)	1.4 (0.2)	0.006*	< 0.001*	0.06
Iron (µg/dL)	63.56 (50.3)	90.09 (35.1)	88.89 (42.0)	0.013*	0.014*	0.99
Hb (g/dL)	8.18 (1.8)	9.7 (1.2)	14.2 (1.9)	< 0.001*	< 0.001*	< 0.001*
Hct (%)	24.85 (5.7)	29.25 (3.5)	43.51 (5.2)	< 0.001*	< 0.001*	< 0.001*
HbF (%)	13.33 (7.7)	18.19 (7.6)	0.91 (0.2)	0.001*	< 0.001*	< 0.001*
RBC count ($\times 10^6$/µL)	3.44 (3.1)	3.5 (0.7)	4.87 (0.45)	0.98	< 0.001*	< 0.001*
MCV (fL)	86.87 (13.2)	85.05 (10.6)	89.21 (7.6)	0.69	0.53	0.12
MCH (pg)	28.72 (4.9)	28.32 (4.1)	29.21 (2.9)	0.91	0.83	0.54
MCHC (g/dL)	32.98 (1.8)	33.29 (1.5)	32.71 (1.1)	0.62	0.66	0.14
RDW (%)	18.52 (3.8)	17.03 (2.4)	13.68 (1.6)	0.027*	< 0.001*	< 0.001*
TLC ($\times 10^3$/µL)	12.88 (7.1)	8.22 (2.9)	7.06 (1.4)	< 0.001*	< 0.001*	0.38
Neutrophils (%)	59.36 (15.8)	54.59 (11.3)	50.56 (12.8)	0.23	0.005*	0.29
Lymphocytes (%)	29.83 (14.5)	35.54 (11.1)	36.29 (10.7)	0.08	0.029*	0.95
Monocytes (%)	9.04 (3.2)	7.58 (3.5)	6.29 (3.0)	0.09	< 0.001*	0.12
Eosinophils (%)	1.42 (1.4)	1.87 (1.6)	2.99 (1.9)	0.44	< 0.001*	0.004*
Basophils (%)	0.36 (0.2)	0.43 (0.2)	0.44 (0.2)	0.39	0.3	0.99
Platelets ($\times 10^3$/µL)	301.23 (182.2)	252 (112)	273.3 (58.9)	0.15	0.52	0.66
LMR	3.67 (2.5)	6.81 (5.6)	7.57 (4.9)	0.006*	< 0.001*	0.69
NMR	8.05 (5.6)	10.67 (9.9)	10.57 (7.7)	0.29	0.29	0.99
NLR	2.77 (1.8)	1.92 (1.8)	1.54 (0.7)	0.022*	< 0.001*	0.41
SII	892.51 (1017.5)	522.26 (773.5)	427.54 (244.9)	0.048*	0.006*	0.79

T. Bil total bilirubin, *D. Bil* direct bilirubin, *I. Bil* indirect bilirubin, *ALP* alkaline phosphatase, *ALT* alanine transaminase, *AST* aspartate transaminase, *AG ratio* albumin-globulin ratio, *Hb* hemoglobin, *HbF* fetal hemoglobin, *Hct* hematocrit, *RBC count* red blood cell count, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *RDW* red-cell distribution width, *TLC* total leucocyte count, *LMR* lymphocyte-monocyte ratio, *NMR* neutrophil-monocyte ratio, *NLR* neutrophil–lymphocyte ratio, *SII* systemic immune-inflammatory index

*Significance at $p < 0.05$; a versus b denotes p-value compared between crisis and steady state cases; a vs c denotes p-value compared between crisis state and healthy control groups; b vs c denotes p-value compared between steady state cases and the healthy control group

When compared between the crisis and steady state cases, a significant reduction was observed for Hb (8.18 g/dL, $p < 0.001$), Hct (24.85%, $p < 0.001$), HbF (13.33%, $p = 0.001$), and LMR (3.67, $p = 0.006$), values in the crisis group. The cases enrolled under crisis state depicted a significant increase in RDW (18.52%. $p = 0.027$), total leucocyte count (TLC) (12.88 × 10^3/µL, $p < 0.001$), NLR levels (2.77, $p = 0.022$), and SII (892.51, $p = 0.048$).

Table 2 Comparison of mean (SD) of the red cell indices among the study groups (N = 140)

Parameters (units)	Crisis state[a] (n = 40)	Steady state[b] (n = 46)	Control group[c] (n = 54)	p-values		
				a vs b	a vs c	b vs c
RDW/Hct	0.8 (0.3)	0.59 (0.1)	0.32 (0.1)	<0.001*	<0.001*	<0.001*
MI	32.31 (15.68)	25.56 (7.46)	18.49 (2.48)	0.004*	<0.001*	0.001*
RDWI	609.09 (395.61)	432.80 (131.96)	251.54 (33.71)	0.001*	<0.001*	<0.001*
GKI	192.24 (121.71)	129.86 (38.62)	76.88 (11.46)	<0.001*	<0.001*	0..001*
EI	52.51 (36.38)	50.02 (16.33)	40.51 (9.15)	0.86	0.029*	0.09
EFI	39.13 (17.85)	29.53 (12.58)	9.62 (8.1)	0.002*	<0.001*	<0.001*
RI	6.78 (3.43)	5.02 (1.1)	2.84 (0.46)	<0.001*	<0.001*	<0.001*
SI	10.72 (5.24)	8.55 (2.67)	6.05 (0.82)	0.006*	<0.001*	0.001*
SLI	2322.13 (1045.42)	2149.52 (780.44)	2376.98 (607.69)	0.59	0.94	0.34
HHI	38.61 (14.78)	31.49 (5.65)	21.9 (2.1)	0.001*	<0.001*	<0.001*
SirI	58.89 (15.91)	52.38 (11.67)	41.55 (6.32)	0.026*	<0.001*	<0.001*

MI Mentzer index, *RDWI* RDW index, *GKI* Green and King index, *EI* Ehsani index, *EFI* England and Fraser index, *RI* Ricerca index, *SI* Srivastava index, *HHI* Huber–Heklotz index, *SirI* Sirdah index

*Significance at $p < 0.05$; a vs b denotes *p*-value compared between crisis and steady state cases; a vs c denotes *p*-value compared between crisis state and healthy control groups; b vs c denotes *p*-value compared between steady state cases and the healthy control group

Table 2 decodes the mean (SD) comparison of the red cell indices in the study groups. Compared to the control group, both cases depicted a significant rise in RDW/Hct, MI, RDWI, GKI, EFI, RI, SI, HHI, and SirI values. Compared to the steady state cases, the crisis state individuals reported an increase in all indices except for EI and SLI. Similarly, except for EI, and SLI, other indices values were higher in steady state cases than in the control group.

The ROC analysis in the cases with cut-off values for the parameters with an AUC of more than 0.6 is delineated in Table 3 and shown in Fig. 1. The AUC observed by blood Hb was 0.78 ($p < 0.001$), and Hct was 0.77 ($p < 0.001$) (shown in Fig. 1a). The AUC for red cell indices like GKI and RI were more than 0.7 ($p = 0.001$) (shown in Fig. 1b). Serum albumin reflected the highest AUC of 0.824 ($p < 0.001$) for crisis events in cases, followed by serum iron levels (0.785, $p < 0.001$) (shown in Fig. 1c). The sensitivity, specificity, PPV, and NPV were calculated for crisis events in cases for both the extrapolated cut-off values from the ROC curve and observed mean values for the parameters in the steady state cases to check which could be used as a cut-off value for the parameter with better sensitivity.

The sensitivity and specificity of the parameters are illustrated in Table 3 and Fig. 1. The parameters with an AUC of more than 0.6 were considered for calculating the cut-off value for the crisis event in the cases. The sensitivity and specificity for crisis events were calculated for the cut-off values and were compared to the mean value of the parameters calculated for the steady state group, as shown in Tables 1 and 2. It was observed that the sensitivity of the crisis event to occur was higher for the mean values of Hb (87.5% vs. 77.5%), Hct (85% vs. 77.5%), lymphocytes (67.5% vs. 65%), LMR (95% vs. 75%), total protein (75% vs. 70%), albumin (85% vs. 77.5%), iron (85% vs. 80%), and RI (72.5% vs. 70%) values as compared to the ROC extrapolated cut-off values. Whereas for other parameters, the sensitivity is higher for the calculated cut-off values. The LMR depicted a sensitivity of 95% with a PPV of 53.5% at a mean cut-off value of 6.81. Similarly, the sensitivity and PPV values were respectively 85% and 64.2% for albumin at a mean value of 4.47 g/dL. The sensitivity for Hb at 9.72 g/dL was 87.5%, and that for Hct at 29.25% was 85% for the crisis event to occur.

As hypothesized, the reference interval in sickle cell disease patients must be revised. Hence, 95th percentile values in steady state were the baseline for comparison of the quantitative variables in crisis state cases. The mean, the 2.5th and 97.5th percentile distribution, has been delineated in Table 4. The table illustrates the ranges in the three study groups. The variations in the parameters' lower (2.5th percentile) and upper (97.5th percentile) values were observed. The 95th percentile range for serum potassium in steady state cases was 3.75 to 6.09 mmol/L as compared to the control group (3.44–5.3 mmol/L). Similarly, the range was higher for total, direct, and indirect bilirubin (I. Bil). The 97.5th percentile value for ALP (186 U/L) and GGT (240 U/L) were higher in steady state cases than in the control group (117 and 42 U/L, respectively). Serum albumin, globulin, and AGR reported a broader range in 95% population of the steady state cases. Blood Hb ranged from 7.51 to 12.17 g/dL in steady state cases compared to 10.4–17.7 g/dL in healthy individuals. The upper limit of RDW in the steady state was 25.56%, whereas it was 19.5% in the control group. The 2.5th percentile value of the neutrophil value was

Table 3 ROC analysis for quantitative variables in depicting the sensitivity and 1-specificity for occurrence of crisis state in the cases (N = 86)

Parameters	AUC	p-value	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Mean value as Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Urea (mg/dL)	0.657	0.012	15.5	70	56.5	58.3	68.4	15.63	70	56.5	58.3	68.4
AST (U/L)	0.719	<0.001	34.5	75	56.5	60	72.2	35.87	67.5	63	61.4	69
ALP (U/L)	0.664	0.009	94.5	75	52.2	57.7	70.6	97.43	67.5	52.2	55.1	64.9
GGT (U/L)	0.669	0.007	19.50	70	65.2	63.6	71.4	33.7	47.5	73.9	61.3	61.8
Total protein (g/dL)	0.711	0.001	7.85	70	71.7	68.3	73.3	8.09	75	54.3	58.8	71.4
Albumin (g/dL)	0.824	<0.001	4.35	77.5	71.7	70.5	78.6	4.47	85	58.7	64.2	81.8
Iron (µg/dL)	0.785	<0.001	71.7	80	71.7	71.1	80.5	90.09	85	39.1	54.8	75
Hb (g/dL)	0.78	<0.001	9.05	77.5	65.2	66	76.9	9.72	87.5	39.1	55.6	60.9
Hct (%)	0.77	<0.001	28.15	77.5	60.9	63.3	75.7	29.25	85	45.7	57.6	77.8
HbF (%)	0.683	0.004	18.55	75	54.3	58.8	71.4	18.19	72.5	56.5	59.2	70.3
RBC count (×10⁶/µL)	0.696	0.002	3.49	72.5	47.8	54.7	66.7	3.5	72.5	47.8	54.7	66.7
TLC (×10³/µL)	0.694	0.002	7.86	70	58.7	59.6	69.2	8.22	67.5	60.9	60	68.3
Lymphocytes (%)	0.65	0.017	33.4	65	52.2	54.2	63.2	35.54	67.5	43.5	50.9	60.6
LMR	0.704	0.001	4.77	75	52.2	57.7	70.6	6.81	95	28.3	53.5	86.7
SII	0.62	0.057	363.72	75	52.2	57.7	70.6	522.25	72.5	56.5	59.2	70.3
RDW/Hct	0.685	<0.001	0.57	85	54.3	61.8	80.6	0.59		100		53.5
MI	0.657	0.012	23.51	70	41.3	50.9	61.3	25.56		100		53.5
RDWI	0.685	0.003	474.13	67.5	65.2	62.8	69.8	432.8	67.5	50	54	63.9
GKI	0.717	0.001	137.03	70	56.5	58.3	68.4	129.86	70	52.2	56	66.7
EI	0.605	0.095	45.75	62.5	39.1	47.2	54.5	50.03	62.5	45.7	50	58.3
EFI	0.682	0.004	29.09		100		53.5	29.53	67.5	43.5	50.9	60.6
RI	0.711	0.001	5.17	70	54.3	57.1	67.6	5.02	72.5	52.2	56.9	68.6
SI	0.645	0.021	8.16	70	52.2	56	66.7	8.55	67.5	52.2	55.1	64.9
SLI	0.538	0.54	1563.49	70	30.4	46.7	53.8	2149.52	52.5	45.7	45.7	52.5
HHI	0.689	0.003	31.37	70	52.2	56	66.7	31.49	70	52.2	56	66.7
SirI	0.634	0.032	50.11	70	41.3	50.9	61.3	52.38	65	43.5	50	58.8

ROC receiver operating characteristics curve, *AUC* area under curve, *PPV*- positive predictive value, *NPV* negative predictive value, *ALP* alkaline phosphatase, *AST* aspartate transaminase, *Hb* hemoglobin, *HbF* fetal hemoglobin, *Hct* hematocrit, *RBC count* red blood cell count, *RDW* red-cell distribution width, *TLC* total leucocyte count, *LMR* lymphocyte-monocyte ratio, *SII* systemic immune-inflammatory index, *MI* Mentzer index, *RDWI* RDW index, *GKI* Green and King index, *EI* Ehsani index, *EFI* England and Fraser index, *RI* Ricerca index, *SI* Srivastava index, *HHI* Huber–Heklotz index, *SirI* Sirdah index
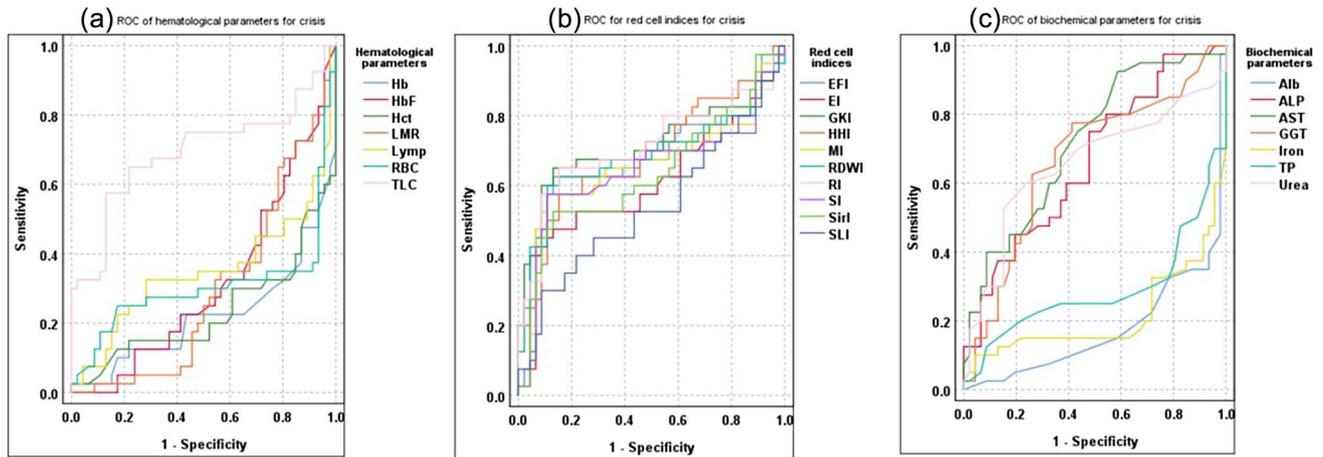
Fig. 1 ROC analysis for quantitative variables for crisis state in cases. *ROC* receiver operating characteristics curve, *Hb* hemoglobin, *HbF* fetal hemoglobin, *Hct* hematocrit, *RBC* count red blood cell count, *RDW* red-cell distribution width, *TLC* total leucocyte count, *LMR* lymphocyte-monocyte ratio, *SII* systemic immune-inflammatory index, *MI* Mentzer index, *RDWI* RDW index, *GKI* Green and King index, *EI* Ehsani index, *EFI* England and Fraser index, *RI* Ricerca index, *SI* Srivastava index, *HHI* Huber–Heklotz index, *SirI* Sirdah index, *Alb* albumin, *ALP* alkaline phosphatase, *AST* aspartate transaminase, *TP* total protein

25.82% compared to 4.34% in the control group. The range for platelet count was wider (108.35 to $543 \times 10^3/\mu L$) in 95% of the steady state cases than in the control group. Similarly, it was true for NLR, HbF values, and the red cell indices in steady state cases.

The scatter plot reflecting the correlation of Hb, HbF levels, and severity scores with the red cell indices in the cases is shown in Figs. 2, 3, 4. All red cell indices except EI and SLI depicted a significant inverse relation with Hb levels ($p < 0.001$) (shown in Fig. 2a–j). Similarly, these parameters correlated negatively with HbF levels in the cases but were insignificant ($p > 0.05$) (shown in Fig. 3a–j). On the contrary, the severity score of the patients with SCD recorded an insignificant but positive correlation with the red cell indices except for SLI (shown in Fig. 4a–j).

Discussion

Individuals with homozygous, HbSS genotype are prone to vascular occlusions, coagulation cascade activation, and ischemic injuries, resulting in vaso-occlusive crisis and thromboembolic events. Initially, these events are often presented as hemolytic or painful crises that eventually progress to fatal conditions like acute chest syndrome, aplastic crisis, and hemorrhagic events. Thus, a baseline of the blood parameters in the steady state rather than healthy control group were evaluated based on which early biomarkers could be identified that could assess the progression of the disease course would be essentially beneficial. The mean values of the parameters like total protein, albumin, iron, Hb, Hct, LMR, and RI depicted higher sensitivity for crisis events.

Although SCD is a disorder of red cells, overall alteration in the serum parameters associated with the disorder's pathophysiology could not be ignored. Olawale et al. study showed significantly reduced urea and creatinine ($p < 0.001$) levels in HbS cases than HbA children [15]. On the contrary, Ko et al. study reported that significant increase in urea ($p = 0.04$) and creatinine ($p = 0.0001$) levels in SCD cases than the control group [16]. The findings emphasized that RFT markers should be monitored regularly for early assessment of kidney injury. The baseline serum urea and creatine were significantly lower in SCD steady state than in the control group (Table 1). These levels were elevated in the crisis state compared to the steady state. The rise in serum urea was comparable to that of the control group ($p = 0.98$), but the increase in serum creatinine was not ($p = 0.038$). The result indicated that the patients might have compromised kidney function, justifying the rise in serum urea levels above the baseline (steady state level), although the mean value was comparable to the healthy control group. Lower levels of serum creatinine might have been due to the overall poor built and reduced muscle mass due to nutritional cause or chronic anemia. The baseline serum urea levels in SCD cases may thus be redefined to the 95% values of 6.53–32.6 mg/dL (Table 4) with a mean cut-off value of 15.63 mg/dL with 70% sensitivity for crisis event (Table 3).

The steady state cases depicted low serum sodium but raised potassium levels than the control group, possibly due to altered fluid homeostasis. Ko et al. study observed a significant rise in serum potassium in SCD cases but no differences in sodium levels [16]. Anti-Boasiako et al. study depicted a substantial decrease in serum sodium but an elevation in potassium levels in SCD cases compared to

Table 4 The 95 percentile values of the quantitative variables in the study groups (N = 140)

Parameters (units)	Crisis state	Steady state	Control group
	Mean (2.5–97.5th percentile range)	Mean (2.5–97.5th percentile range)	Mean (2.5–97.5th percentile range)
Urea (mg/dL)	20.52 (4.9 -95.43)	15.63 (6.53–32.6)	20.89 (11–39)
Creatinine (mg/dL)	0.75 (0.35–2.75)	0.68 (0.47–0.98)	0.89 (0.72–1.05)
Uric acid (mg/dL)	5.37 (2.5–16.89)	5.09 (2.94–7.69)	5.6 (2.8–9.8)
Sodium (mmol/L)	136.35 (126.05–144)	137.98 (132.35–143.65)	140.56 (137–144)
Potassium (mmol/L)	4.36 (3.62–5.72)	4.58 (3.75–6.09)	4.24 (3.44–5.3)
Chloride (mmol/L)	100.95 (84.2–112.98)	102.57 (97–107)	103.44 (99–106)
T. Bilirubin (mg/dL)	3.24 (0.61–19.59)	2.51 (1.02–5.21)	0.89 (0.35–2.02)
D. Bilirubin (mg/dL)	1.01 (0.16–9.79)	0.54 (0.23–2.11)	0.18 (0.08–0.41)
I. Bilirubin (mg/dL)	2.2 (0.45–9.85)	1.98 (0.79–4.38)	0.69 (0.13–1.7)
AST (U/L)	54.25 (16.2–170)	35.87 (17.18–87.27)	27.89 (15–92)
ALT (U/L)	27.93 (7.08–73)	22.33 (5.53–71)	25.63 (9–71)
ALP (U/L)	136.73 (51.28–523.13)	97.43 (46–186)	90.96 (55–117)
GGT (U/L)	47.73 (8–249.5)	33.7 (6.18–240)	20.74 (12–42)
Total protein (g/dL)	7.47 (5.6–9.39)	8.09 (7.1–9.08)	7.89 (7–8.9)
Albumin (g/dL)	3.81 (2.22–4.99)	4.47 (2.9–5.0)	4.59 (4.1–5.3)
Globulin	3.66 (2.4–7.17)	3.61 (2.5–5.07)	3.3 (2.7–3.9)
AG ratio	1.11 (0.31–1.62)	1.29 (0.58–1.99)	1.4 (1.07–1.81)
Iron (µg/dL)	63.56 (22.03–243.6)	90.09 (38.17–221.53)	88.89 (34–201)
Hb (g/dL)	8.18 (4.31–12.38)	9.72 (7.51–12.17)	14.26 (10.4 -17.7)
Hct (%)	24.85 (13.37–41.54)	29.25 (22.54–36.1)	43.51 (32.2–53.5)
HbF (%)	13.3 (0.8–25.7)	18.19 (0.8–28.7)	0.91 (0.8–1.6)
RBC count ($\times 10^6$/µL)	3.44 (1.16–21.29)	3.5 (2.2–5.49)	4.87 (3.79–5.5)
MCV (fL)	86.87 (62.44–116.43)	85.05 (57.22–102.63)	89.21 (65.4–107.9)
MCH (pg)	28.72 (19.91–37.66)	28.35 (17.01–37.41)	29.21 (21.3–35.8)
MCHC (g/dL)	32.99 (27.65–37.17)	33.29 (29.36–36.86)	32.71 (30.3–34.7)
RDW (%)	8.53 (13.41–28.96)	17.03 (13.27–25.56)	13.68 (12.1–19.5)
TLC ($\times 10^3$/µL)	12.88 (4.39–35.36)	8.22 (3.86–16.98)	7.07 (4.09–11.3)
Neutrophils (%)	59.36 (25.21–81.3)	54.59 (25.82–76.64)	50.56 (4.34–71.2)
Lymphocytes (%)	29.83 (12.34–62.59)	35.54 (8.35–62.27)	36.29 (2.9–53.8)
Monocytes (%)	9.04 (3.1–16)	7.58 (1.5–13.93)	6.29 (0.44–12.8)
Eosinophils (%)	1.42 (0–7.71)	1.87 (0.3–7.07)	2.99 (0.11–7.8)
Basophils (%)	0.36 (0.1–0.99)	0.43 (0–0.88)	0.44 (0.04–1.1)
Platelets ($\times 10^3$/µL)	301.23 (59.6–771)	252 (108.35–543)	273.33 (188–422)
LMR	3.67 (1.2–15.69)	6.81 (0.65–24.48)	7.57 (1.89–19.68)
NMR	8.05 (2.45–26.22)	10.67 (2.53–46.79)	10.57 (3.01–41.88)
NLR	2.77 (0.4–6.29)	1.92 (0.42–11.002)	1.54 (0.77–3.56)
SII	892.51 (61.45–4194.02)	522.25 (66.41–4686.82)	427.54 (177.24–1278.20)
RDW/Hct	0.80 (0.38–1.91)	0.59 (0.38–0.87)	0.32 (0.23–0.6)
MI	32.31 (4.05–100.95)	25.56 (10.82–46.49)	18.49 (13.24–23.19)
RDWI	609.09 (101.69–2569.39)	432.8 (206.93–828.37)	251.54 (200.33–347.89)
GKI	192.24 (63.04–795.17)	129.86 (63.82–254.29)	76.88 (60.0–111.44)
EI	52.51 (0–104.65)	50.03 (3.38–80.35)	40.51 (16.0–58.4)
EFI	39.13 (2.15–89.83)	29.53 (1.98–59.46)	9.62 (4.8–28.71)
RI	6.78 (1.24–22.1)	5.02 (2.79–8.1)	2.84 (2.23–3.96)
SI	10.72 (1.29–32.62)	8.55 (3.23–16.38)	6.05 (4.31–7.59)
SLI	2322.13 (825.79–5107.07)	2149.52 (566.89–3870.53)	2376.98 (911.04–4167.98)
HHI	38.61 (20.95–108.45)	31.49 (20.3–45.59)	21.90 (18.79–27.91)
SirI	58.89 (26.96–101.95)	52.38 (24.42–77.87)	41.55 (28.96–52.91)

T. Bil total bilirubin, *D. Bil* direct bilirubin, *I. Bil* indirect bilirubin, *ALP* alkaline phosphatase, *ALT* alanine transaminase, *AST* aspartate transaminase, *AG ratio* albumin-globulin ratio, *Hb* hemoglobin, *HbF* fetal hemoglobin, *Hct* hematocrit, *RBC count* red blood cell count, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *RDW* red-cell distribution width,

Table 4 (continued)

TLC total leucocyte count, *LMR* lymphocyte-monocyte ratio, *NMR* neutrophil-monocyte ratio, *NLR* neutrophil–lymphocyte ratio, *SII* systemic immune-inflammatory index, *MI* Mentzer index, *RDWI* RDW index, *GKI* Green and King index, *EI* Ehsani index, *EFI* England and Fraser index, *RI* Ricerca index, *SI* Srivastava index, *HHI* Huber–Heklotz index, *SirI* Sirdah index

the healthy control group [17]. Similar findings were also reported by Mansoor et al. and Pandey et al. studies [18, 19]. Chronic subclinical level hemolysis might be an explainable cause for elevated serum potassium levels in steady state cases. However, a gross decrease in serum sodium was evident in the crisis state, but the variation in serum potassium was not apparent. The reason resides in extensive hemolysis of the red cell membrane and leakage of intracellular potassium due to the destruction of the red cell membrane in a crisis state [17, 18]. The revised 95% reference interval in steady state cases observed in this study for serum sodium was 132.35–143.65 mmol/L, and that for serum potassium was 3.75–6.09 mmol/L. The lower range of serum sodium was lower than the control group, whereas the serum potassium was higher (Table 4).

Pandey et al. found a gross rise in bilirubin levels and liver enzymes like AST, ALT, and ALP [19]. Obi et al.'s findings also depicted a significant elevation of liver enzymes,mes including GGT, in the SCD cases than the control subjects,cts reflecting an ongoing ischemic injury and vascular crisis events in the liver [20]. True to the fact of chronic hemolysis, serum total bilirubin (T. Bil) is expected to be at a higher range in the steady state SCD cases (1.02–5.21 mg/dL) than the control group (0.35–2.02 mg/dL) (Table 4). However, the direct bilirubin was significantly raised in crisis cases than in the steady state cases ($p = 0.038$, Table 1), suggesting an activated bilirubin conjugation process generated by heme breakdown during red cell destruction. The liver enzyme levels in the steady state were comparable to that in the control group (Table 1), indicating a normally functioning liver in the study population. However, the elevated AST and ALP levels in crisis events could have been due to ischemic injuries in bones, skeletal muscles, cardiac muscles, or other tissues that release these enzymes consequent to the thromboembolic events as reflected by Obi et al. study [20]. The observed 95 percentile range in the steady state and the control group were almost similar except for ALP and GGT (Table 4), and the mean cut-off value reported lower sensitivity for the crisis state (Table 3).

Obi et al. study reported a significant fall in total protein and albumin in SCD cases ($p < 0.05$) compared to the control individuals due to a reduction in short-lived serum proteins and loss of albumin in ascitic fluid [20]. On the contrary, Pandey et al. showed grossly elevated serum total proteins in cases than the control group ($p < 0.001$) [19]. The present study findings reflected that the serum total protein, albumin, and AGR values in steady state were at par with the control group (Table 1), which further approves a healthy liver in

these patients. But the levels were significantly decreased in the crisis state than the steady state. The sensitivity of total protein and albumin (75% and 85%, respectively) for mean cut-off values of 8.09 and 4.47 g/dL were better than the ROC-evaluated cut-off values of 7.85 and 4.35 g/dL (Table 3, Fig. 1c). The new disease specific reference interval for serum albumin was broader (2.9–5.0 g/dL) than the control group (4.1–5.3 g/dL). The lowering of these proteins might result from the acute phase reaction owing to inflammatory state due to aggravated ischemic injury during vaso-occlusive crisis event. However, a raised serum globulin in crisis group and a wider 95% reference interval in steady state cases as compared to control group indicated a state of chronic inflammation.

Studies have reported elevated serum ferritin in SCD individuals, which is considered the marker for the body's iron store [21, 22]. Akodu et al. showed a significant increase in serum ferritin ($p = 0.001$) in HbSS cases than in HbAA individuals, but the serum iron levels were comparable [22]. Makulo et al. reported raised serum ferritin in nearly 20% of SCD cases, of which about 93% had serum iron within the normal range. The findings implied higher rates of iron stores in the reticuloendothelial system due to ongoing red cell destruction [21]. Serum iron levels did not differ in the steady state cases compared to the control group. However, iron levels were grossly reduced in the crisis state, which justifies the overutilization of iron for erythropoiesis to compensate for ongoing hemolysis. Serum iron < 90.09 μg/dL reported a sensitivity of 85% for crisis events (Table 3, Fig. 1c). However, serum ferritin was not evaluated in the present study, which could have provided a better understanding of the iron reserve in the study population.

Various studies observed reduced Hb, Hct levels, and RBC count in SCD cases than the control group [6, 7, 9]. Evident of the pathophysiology of hemolysis in SCD, Hb, Hct values, and RBC count were significantly lower, and HbF values were elevated in cases compared to the control group. In agreement with the Khaled et al. study, the values in the present study were further decreased during the crisis, although the RBC count was not [9]. On the contrary, Feugray et al. study depicted no change in Hb and Hct levels between the 72 steady state and 26 crisis state cases, but the RBC count was lower in the crisis group [23]. The 95% range for Hb (7.51–12.17 g/dL) and RBC count ($2.2-5.49 \times 10^6$/μL) were relatively broader in steady state cases, whereas the Hct range was lowered to 22.54–36.1% (Table 4). The sensitivity below 9.72 g/dL Hb, 29.25% Hct, and 3.5×10^6/μL was 87.5%, 85%, and 72.5%, respectively (Table 3). Feugray

Fig. 2 Scatter plot reflecting the correlation of the red cell indices with Hb in cases. *Hb* hemoglobin, *MI* Mentzer index, *RDWI* RDW index, *GKI* Green and King index, *EI* Ehsani index, *EFI* England and Fraser index, *RI* Ricerca index, *SI* Srivastava index, *HHI* Huber–Heklotz index, *SirI* Sirdah index
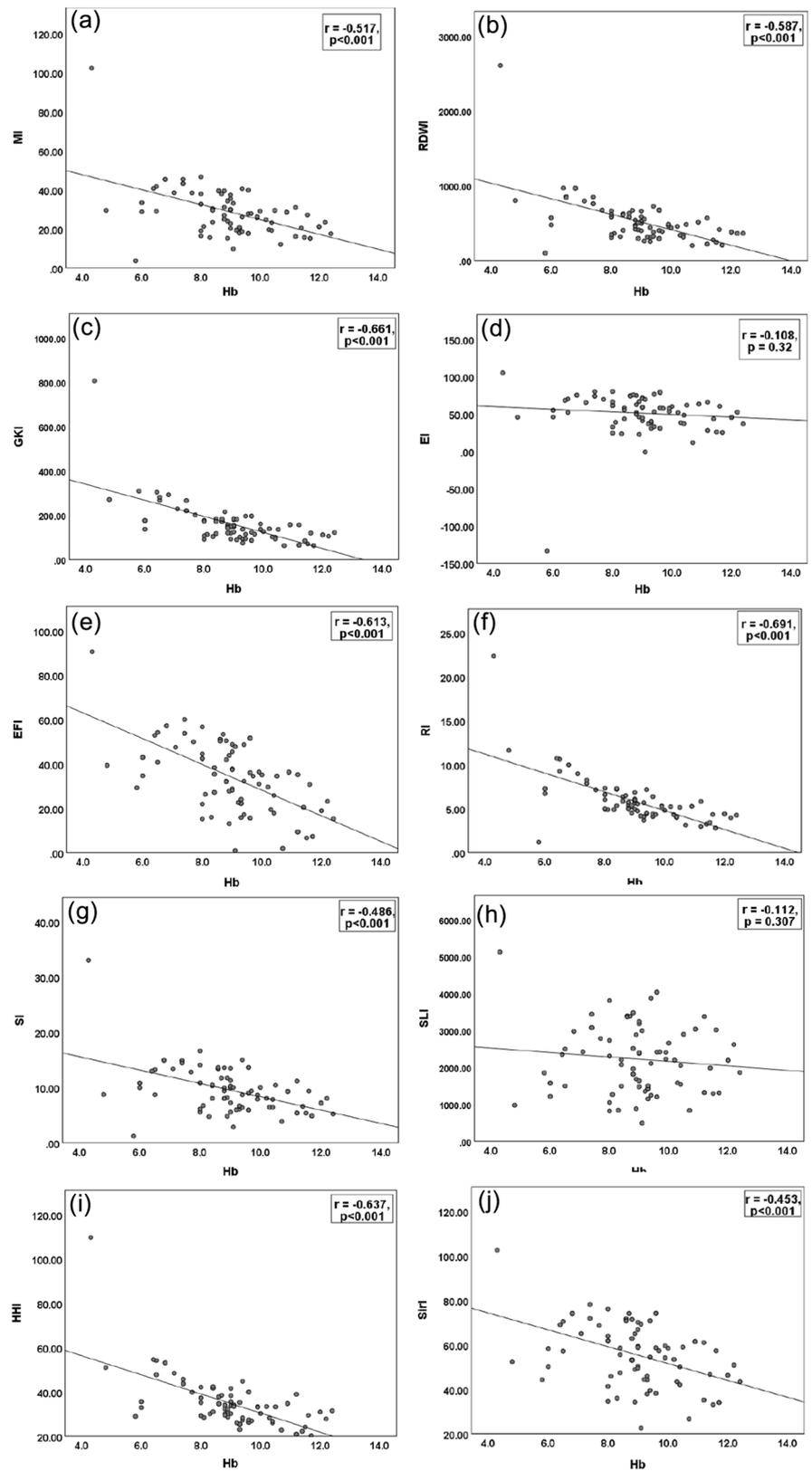
Fig. 3 Scatter plot reflecting the correlation of the red cell indices with HbF in cases. *HbF* fetal hemoglobin, *MI* Mentzer index, *RDWI* RDW index, *GKI* Green and King index, *EI* Ehsani index, *EFI* England and Fraser index, *RI* Ricerca index, *SI* Srivastava index, *HHI* Huber–Heklotz index, *SirI* Sirdah index
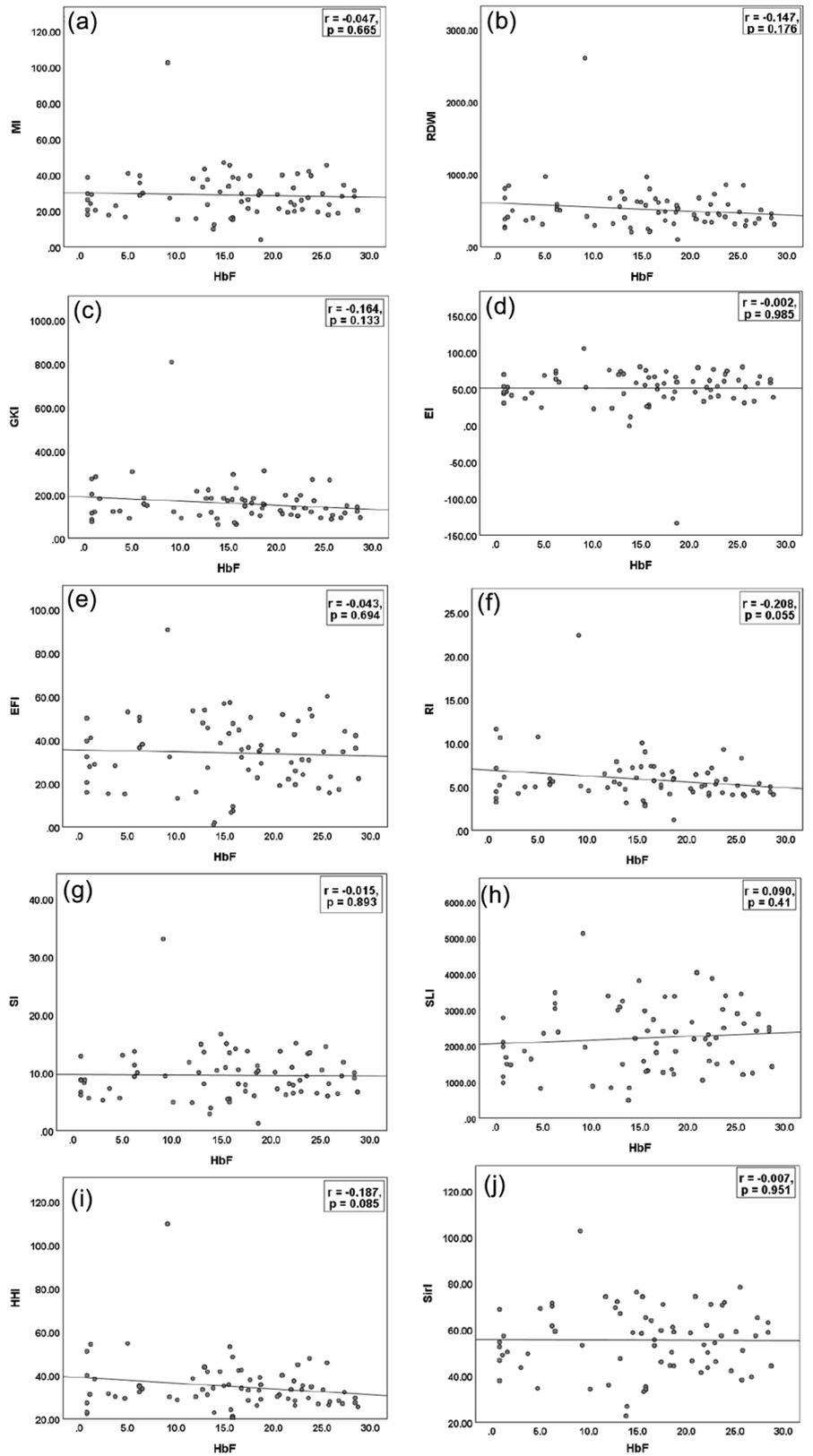
Fig. 4 Scatter plot reflecting the correlation of the red cell indices with severity score in cases. *MI* Mentzer index, *RDWI* RDW index, *GKI* Green and King index, *EI* Ehsani index, *EFI* England and Fraser index, *RI* Ricerca index, *SI* Srivastava index, *HHI* Huber–Heklotz index, *SirI* Sirdah index
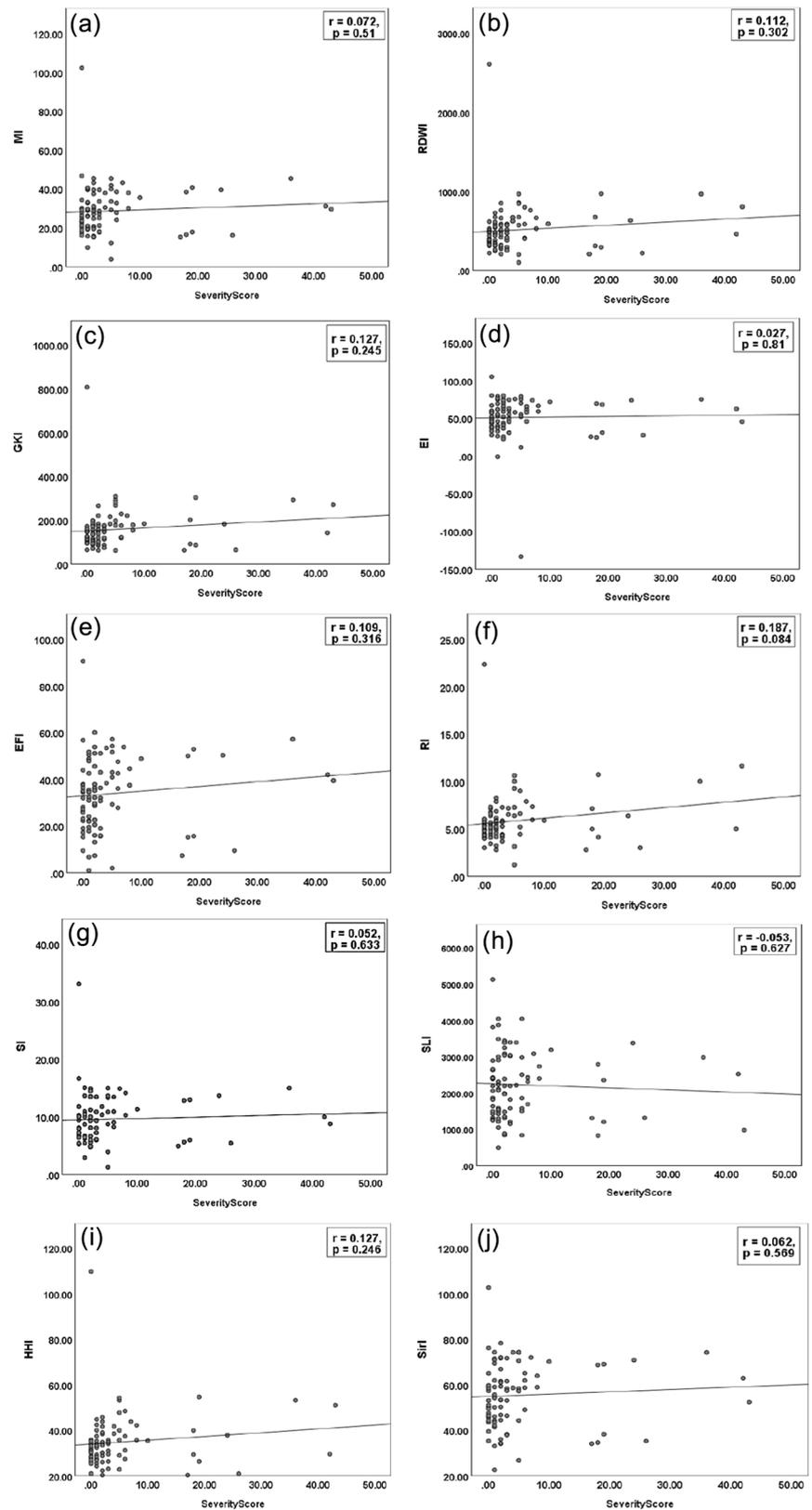
et al. study reported the Hb range to be 8–10.5 gm/dL in steady state and 8–10.1 gm/dL in crisis state cases. The new reference values for Hct in steady state was 23–30.7%, and in crisis state was 23–29%. The respective ranges for RBC counts were 2.67–3.97 and 2.39–3.18×10^{12}/L [23]. The mean (SD) recorded in SCD cases by Khaled et al. study for Hb was 9.10 (2.2 g/dL), Hct was 27.23 (3.84)%, and RBC count was 3.78 (1.31)×10^6/μL [9]. The differences in range could be accorded to the differences in socioeconomic factors, nutritional factors or geographical locations of the two study populations. Our study population belongs to Central India, considered a developing country, whereas the study population included in Feugray et al. and Khaled et al. study belonged to a European country [9, 23]. According to the severity of anemia in SCD cases, the RDW%, RDW/HCT, and RDWI were significantly higher in steady state cases than in the control group. The same was higher in crisis patients than the steady state patients (Table 1). Accordingly, the upper limit of the baseline range in steady state was more elevated than the control group.

The pathophysiology of SCD, besides affecting the RBC deformation and rheological properties, also activates neutrophils, monocytes, and platelets adhesiveness to the capillary walls initiating VOC. The continued process of RBC sickling, microvascular occlusion, and ischemic insult to various tissues creates a chronic inflammatory state [24]. The crisis group enrolled in Feugray et al. study depicted a significant increase in leucocytes, neutrophils, lymphocytes, and monocytes than in steady state SCD cases [23]. Studies have evidenced inflammatory activation, raised c-reactive protein, leucocytes, annexin-1, and other inflammatory markers, during the VOC crisis event [25, 26]. On the contrary, TLC, lymphocyte, and LMR values did not reflect significant variation in steady state cases and the control group (Table 1). However, the LMR values revealed a wider baseline range in the steady state (0.65–24.48, Table 4), and the mean value of 6.81 denoted a sensitivity of 95% for the crisis state (Table 3, Fig. 1a). Significant variations of these parameters in crisis events explain the activation of the varied leucocytes to tackle the crisis state but remained unaltered in steady state cases. Accordingly, the SII values in steady state were comparable to that of the control group, but the baseline range was broader (Table 4), and the mean value of 522.25 depicted a sensitivity of 72.5% for the crisis to occur (Table 3).

SCD patients in this geographical region are often associated with nutritional anemia, iron or B12 deficiency. The present study did not observe any significant differences between the groups in the MCV, MCH, and MCHC values. The observation might have been influenced due to the endemicity of malaria, iron deficiency anemia, and other causes of anemia. Diagnostic evaluation for anemia, such as measurement of serum ferritin, B12, folic acid levels,

and malarial antigen, would have been beneficial in discriminating the cases accordingly. Red cell indices should be evaluated to differentiate the categories accordingly. Very few studies could be found regarding the discrimination ability of the calculated red cell indices for crisis and steady state in SCD patients. Ahmed et al. demonstrated SLI as a screening tool for sickle cell with 78% sensitivity and 92% specificity [27]. Lakhani et al. revealed increased MI and SI in SCD cases than in SCT cases, suggesting iron deficiency in these patients [28]. Khaled et al. study observed significantly raised SLI, RI, SirI, and HH in SCT cases compared to the healthy control group. However, the RDWI, GKI, EF, SI, RI, SirI, and HH values were higher in SCD cases compared to SCT cases ($p < 0.05$). The study suggested SirI and EF indices screen out SCD and SCT with the Youden index (YI) of 87 and 83, respectively [9]. Sahli et al. findings suggested EI, MI, and SLI as the indices for differentiating SCD and SCT [8]. All the red cell indices except for EI and SLI were grossly elevated and recorded a broader baseline range in steady state than the control group (Tables 2 and 4). However, the mean values of GKI (129.86), RI (5.02), and HHI (31.49) recorded a sensitivity close to 70% (Table 3, Fig. 1b). All these parameters showed a significant negative correlation with Hb except for EI and SLI (shown in Fig. 2a–j). Although not significant to the level $p < 0.05$, the indices depicted a positive correlation with the severity score (shown in Fig. 4a–j) and negative influence on HbF (except for SLI) in the cases (shown in Fig. 3a–j). The finding accorded with the pathophysiology of the hemolytic crisis or painful crisis in these patients that immensely affect the red cell morphology and structural integrity. A larger sample size could have provided a better insight regarding the association of these indices with HbF and severity score.

Strength and Limitations

The primary strength of the study is the evaluation of such a wide array of hematological and biochemical parameters. Few published articles have compared such a wide array of blood parameters in crisis and steady state in SCD cases. Of them, only a few derived the red cell indices, inflammatory indices, and the steady-state reference interval. The major limitation of this study was the sample size. A study on a larger cohort of SCD from different areas should be designed for deriving the most sensitive and specific predictor marker. Detailed diagnostic evaluation of associated iron deficiency anemia and other causes of anemia was lacking in the present study. Including different Hb variants would have better characterized the quantitative variables.

Conclusion

The study concluded that steady state cases should be monitored regularly to assess the course of the disease and early affirmation regarding switching to a crisis state. The markers included are routinely performed in all hospital set up. Compared to the steady state, the crisis state cases depicted a significant increase in urea, AST, ALP, RDW%, TLC, inflammatory indices like NLR, SII, and all red cell indices except EI and SLI. A significant reduction was observed for sodium, total protein, albumin, AGR, iron, Hb, Hct, HbF, and LMR. Serial monitoring of Hb, Hct, LMR, total protein, albumin, and iron levels would benefit early assessment of crisis events. Red cell indices like RI, GKI, and HH could be good screening tools for crisis events, provided the cut-off values have been correctly evaluated for that population. The sensitivity of these markers as per the new cut-off derived from the mean values of the steady state cases was more than 70%. The red cell indices depicted inverse relation with Hb and a positive association with severity score. Thus, the laboratory should derive a new disease specific reference interval and cut-off values at the steady state in SCD cases from the population it is catering samples rather than comparing it with healthy individuals or as per the manufacturer's kit insert. This approach in daily practice would benefit SCD patients for early identification and therapeutic intervention.

Data Availability   The data is presently available with the principal investigator (corresponding author). It is not available in the public domain and can be made available to the editor or reviewer against a request letter. The letter shall be reviewed and approved by all the authors and the Head of the Institution, following which it shall be made available.

Declarations

Conflict of interest   The authors have no conflicts of interest to declare.

Ethical Approval   The study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. This study protocol was reviewed and approved by Institute Ethics Committee, AIIMS, Raipur, with approval number 2163/IEC-AIIMSRPR/2022.

Consent to Participate   Written informed consent was obtained from participants to participate in the study.

References

1. Inusa BPD, Hsu LL, Kohli N, Patel A, Ominu-Evbota K, Anie KA, et al. Sickle cell disease—genetics, pathophysiology, clinical presentation and treatment. Int J Neonatal Screen. 2019;5(2):20.

2. Patra PK, Chauhan VS, Khodiar PK, Dalla AR, Serjeant GR. Screening for the sickle cell gene in Chhattisgarh state, India: an approach to a major public health problem. J Community Genet. 2011;2(3):147–51.

3. Patel S, Nanda R, Hussain N, Mohapatra E, Patra PK. Frequency distribution of the methylenetetrahydrofolate reductase polymorphisms in sickle cell hemoglobinopathy-A hospital based study in central India. Clin Epidemiol Glob Health. 2021;10(100669):1–7.

4. Thaker P, Colah RB, Patel J, Raicha B, Mistry A, Mehta V, et al. Newborn screening for sickle cell disease among tribal populations in the states of Gujarat and Madhya Pradesh in India: evaluation and outcome over 6 years. Front Med. 2022;8(731884):1–8.

5. Sundd P, Gladwin MT, Novelli EM. Pathophysiology of sickle cell disease. Annu Rev Pathol. 2019;24(14):263–92.

6. Akinbami A, Dosunmu A, Adediran A, Oshinaike O, Adebola P, Arogundade O. Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria. BMC Res Notes. 2012;1(5):396.

7. Fome AD, Sangeda RZ, Balandya E, Mgaya J, Soka D, Tluway F, et al. Hematological and biochemical reference ranges for the population with sickle cell disease at steady state in Tanzania. Hemato. 2022;3(1):82–97.

8. Sahli CA, Bibi A, Ouali F, Fredj SH, Dakhlaoui B, Othmani R, et al. Red cell indices: differentiation between β-thalassemia trait and iron deficiency anemia and application to sickle-cell disease and sickle-cell thalassemia. Clin Chem Lab Med. 2013;51(11):2115–24.

9. Khaled SAA, Ahmed HA, Elbadry MI, NasrEldin E, Hassany SM, Ahmed SA. Hematological, biochemical properties, and clinical correlates of hemoglobin S variant disorder: a new insight into sickle cell trait. J Hematol. 2022;11(3):92–108.

10. Green R, King R. A new red cell discriminant incorporating volume dispersion for differentiating iron deficiency anemia from thalassemia minor. Blood Cells. 1989;15(3):481–91; discussion 492–495.

11. Ehsani MA, Shahgholi E, Rahiminejad MS, Seighali F, Rashidi A. A new index for discrimination between iron deficiency anemia and beta-thalassemia minor: results in 284 patients. Pak J Biol Sci PJBS. 2009;12(5):473–5.

12. Shine I, Lal S. A strategy to detect beta-thalassaemia minor. Lancet Lond Engl. 1977;1(8013):692–4.

13. Huber A, Ottiger C, Risch L, Regenass S, Hergersberg M, Herklotz R. Thalassämie-syndrome: klinik und diagnose syndromes thalassémiques: clinique et diagnostic. Swiss Med Forum. 2004;8(4):947–52.

14. Sirdah M, Tarazi I, Al Najjar E, Al HR. Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the beta-thalassaemia minor from iron deficiency in Palestinian population. Int J Lab Hematol. 2008;30(4):324–30.

15. Olawale OO, Adekanmbi AF, Sonuga AA, Sonuga OO, Akodu SO, Ogundeyi MM. Assessment of renal function status in steady-state sickle cell anaemic children using urine human neutrophil

gelatinase-associated lipocalin and albumin: creatinine ratio. Med Princ Pract. 2021;30(6):557–62.

16. Ko A, Ic I, So O, Eo DI, Ri E, E O, et al. Renal function of sickle cell subjects in Edo State-Nigeria. Arch Nephrol Urol. 2018;1(1):1–8.

17. Antwi-Boasiako C, Kusi-Mensah YA, Hayfron-Benjamin C, Aryee R, Dankwah GB, Abla KL, et al. Serum potassium, sodium, and chloride levels in sickle cell disease patients and healthy controls: a case-control study at Korle-Bu Teaching Hospital, Accra. Biomark Insights. 2019;14(1177271919873889):1–5.

18. Mansoor F, Bai P, Kaur N, Sultan S, Sharma S, Dilip A, et al. Evaluation of serum electrolyte levels in patients with anemia. Cureus. 2021;13(10):e18417.

19. Pandey S, Sharma A, Dahia S, Shah V, Sharma V, Mishra RM, et al. Biochemical indicator of sickle cell disease: preliminary report from India. Indian J Clin Biochem. 2012;27(2):191–5.

20. Cu O. Enzyme activities of liver function (Biomarkers) in sickle cell anaemic patients attending Sickle Cell Anaemic Centre, Benin City, Edo State, Nigeria. Int J Blood Res Disord. 2020;7(2):1–5.

21. Makulo JR, Itokua KE, Lepira RK, Bundutidi GM, Aloni MN, Ngiyulu RM, et al. Magnitude of elevated iron stores and risk associated in steady state sickle cell anemia Congolese children: a cross sectional study. BMC Hematol. 2019;19(3):1–6.

22. Akodu SO, Diaku-Akinwumi IN, Kehinde OA, Njokanma OF. Serum iron status of under-five children with sickle cell anaemia in Lagos, Nigeria. Anemia. 2013;2013(e254765):1–7.

23. Feugray G, Kasonga F, Grall M, Benhamou Y, Bobée-Schneider V, Buchonnet G, et al. Assessment of reticulocyte and erythrocyte parameters from automated blood counts in vaso-occlusive crisis on sickle cell disease. Front Med. 2022;9(858911):1–8.

24. Nader E, Romana M, Connes P. The red blood cell—inflammation vicious circle in sickle cell disease. Front Immunol. 2020;11(454):1–11.

25. Yildirim N, Unal S, Yalcinkaya A, Karahan F, Oztas Y. Evaluation of the relationship between intravascular hemolysis and clinical manifestations in sickle cell disease: decreased hemopexin during vaso-occlusive crises and increased inflammation in acute chest syndrome. Ann Hematol. 2022;101(1):35–41.

26. Torres LS, Okumura JV, Silva DGH, Mimura KKO, Belini-Júnior É, Oliveira RG, et al. Inflammation in sickle cell disease: differential and down-expressed plasma levels of annexin A1 protein. PLoS ONE. 2016;11(11):e0165833.

27. Ahmed HA, Khaled SAA, Fahmy EM, Mohammed NA, Mahmoud HFF. Significance of the mathematically calculated red cell indices in patients with qualitative and quantitative hemoglobinopathies. Egypt J Int Med. 2022;34(1):1–9.

28. Lakhani J, Kalaria T, Sanghavi A, Chordiya R. A study of blood indices in Sickle cell disorder. Ann Trop Med Public Health. 2020;23(23):1–11.