Health Science Reports

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ORIGINAL RESEARCH OPEN ACCESS

Inflammation and Iron Profile in Children With Sickle Cell Disease in Cameroon: Frequency and Associated Factors, an Analytical Cross-Sectional Study

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Received: 18 April 2024 | **Revised:** 23 January 2025 | **Accepted:** 15 April 2025

Funding: The authors received no specific funding for this work.

Keywords: associated factors | inflammation | iron profile | sickle cell anemia

ABSTRACT

Background and Aims: Sickle Cell Disease is a chronic inflammatory disease that could be aggravated by exposure to variable factors such as infections, hemolysis, oxidative stress and so forth. that could preciptate variable acute or end-organ manifestation. However, the degree of inflammation could vary in individuals. Thus this study evaluates the inflammatory state (hs-CRP, IL6, ferritin) of children living with SCD and the associated factors.

Methods: We conducted an analytical cross-sectional study for 03 months. The patients included were those from the Central Hospital of Yaounde and the Regional Hospital of Bafoussam in which they are regularly monitored and/or interned in the Hematology department. The exploration of inflammation was made by determining hs-CRP, IL6, and ferritin concentrations. The hematological parameters and iron profile were evaluated using standard methods. Statistical analyses of the data were carried out using the statistical software R version 4.1.1. from which logistic regression analyses according to univariate and multivariate models made it possible to identify factors associated with inflammation in patients.

Results: Hundred and forty-nine SCD patients were included in the study. The frequency of inflammation in the population was 42.3%. Hyperferritinemia was significantly greater ($p < 0.001$) in patients with inflammation compared to the non-inflammatory patients (96.8% and 76.7% respectively). Patients with inflammation showed a significant elevation of iron parameters ($p < 0.05$). In addition, ferritin and IL6 elevation were associated with inflammation during sickle cell disease, respectively (OR = 4.96; 95% CI [1.15–36.42]; $p = 0.056$) and (OR = 6.23; 95% CI [1.43–45.96]; $p = 0.030$).

Conclusion: The elevated iron in plasma is an effect of inflammation in sickle cell patients. Thus, inflammation constitutes a significant and significant factor in worsening the pathophysiology of sickle cell disease. Hence the need of controlling inflammation and iron in the latter is necessary.

1 | Introduction

Sickle cell disease is one of the world's most common hereditary genetic disorders, causing an abnormal presence of hemoglobin (HbS) in the blood. The consequence of this hemoglobin anomaly is the occurrence of acute and chronic complications including vaso-occlusive crises, acute anemia, splenic sequestrations, and erythroblastopenia among others [1, 2]. It is estimated that around 312,000 sickle cell SS births occur each year worldwide, the vast majority of which (236,000 births) come from sub-Saharan Africa [3]. It is a disease with high inflammatory potential and constitutes a real public health problem.

Sickle cell patients are exposed to various factors causing inflammation, including anoxia causing sickling of sickle cells, frequent hemolysis and vasoocclusive crises in situations of tissue hypooxygenation [4]. Other sources of inflammation have been described in sickle cell patients such as recurrent infections, particularly in children, and significant oxidative stress caused by an imbalance of free radicals-antioxidants in favor of free radicals. However, during the inflammatory reaction, we witness a hyperproduction of pro-inflammatory cytokines including interleukin 6, the production of which induces the synthesis of hepcidin [4]. It is also considered to be the central molecule ensuring iron homeostasis. This is how authors report it as being strongly correlated with serum ferritin levels in anemic patients in inflammatory conditions [5]. In sickle cell patients, the inflammation that causes elevation of serum iron is a risk factor for high oxidative stress [6]. Several studies report an elevation of the precursors of ferric metabolism in correlation with the dysregulation of its metabolism [6]. This is the case of Mangaonkar et al. in 2020 who reported significantly high hepcidin levels in sickle cell patients with iron overload unlike those without it [7]. They also reported positive and significant correlations between hepcidin and ferritin with elevated erythroferone levels in the iron-chelating group [7]. Moreover, Koduri et al. in a review demonstrated iron overload in sickle cell patients positively correlated with inflammation [8]. In addition, Bandeira et al. and other authors reported a significant elevation of pro-inflammatory cytokines in sickle cell patients [9–11].

Children with sickle cell disease do have not strong enough immunity [12]; And coupled with its genetic condition, this is a fertile ground for inflammation to set in. The inflammation in the latter is an aggravating factor in the pathophysiology of the disease because it is involved in the dysregulation of iron metabolism; The iron produced is an important factor in aggravating the pathophysiology of the disease, including oxidative stress and cardiovascular disease [13, 14]. This study, therefore, proposes to describe inflammation in children with sickle cell disease in Cameroon, identify the factors associated with inflammation, and raise the consequences for the patient including the iron profile and the profile of the hemogram.

2 | Materials and Methods

2.1 | Design and Subjects

The population was recruited at the Bafoussam Regional Hospital and the Yaoundé Central Hospital. The sample population consisted of sickle cell patients in the steady state being followed in said hospitals, particularly in the Hematology departments. Steady state was defined for patients whose last CVO dates back more than 6 months.

An estimate of the size of the study population to be included was made based on the arithmetic formula described by Lorentz, taking into consideration the frequency of sickle cell disease in Cameroon (2%–3%) and the frequency of inflammation in Congolese sickle cell children (49%) as reported by Tshilolo et al. [15]. For each participant, socio-demographic and clinical data were obtained.

Homozygous patients (SS) of both sexes, aged 0.5 years and over, consenting to participate in the study and having provided all the required information were included. All sickle cell patients with other pathologies namely hemolytic pathologies including G6PD deficiency, thalassemia, infections, and any other pathology likely to cause inflammation were not included in the study. Moreover, any sickle cell patient on iron supplementation or having undergone a blood transfusion less than 3 months old were not included in this study. A total of 149 homozygous sickle cell patients were finally selected for the study.

2.2 | Sampling and Analysis

A volume of 04 mL of blood was collected from participants separately into EDTA (Ethylene Diamine Tetra Acetic) and dry tubes. Each sample collected was sent to the appropriate laboratory for biological analyses.

The hematological parameters of blood samples were performed on the HumaCount 30TS hematology automaton. The blood count was accompanied by the production of blood smears, particularly for the reticulocyte count and the production of leukocyte formulas using standard stains as described in the Rovanovsky protocols. The prepared slides were read using an 'Irmeco' binocular microscope and the final results obtained were interpreted according to WHO recommendations.

Furthermore, the status of each participant has confirmed by agarose gel electrophoresis determination of hemoglobin followed by the quantification of the different fractions of hemoglobin according to the protocol described by 'Hellabio' *(manufacturer Thessaloniki, Greece)*. Once the migrations had been carried out, the quantification of the different fractions of the hemoglobin was done using a densitometer after staining with red culvert.

Assessment of inflammation in the population was made by determining the CRP concentration by Ultrasensitive technique using the specific PA54 protein analyzer and Genrui brand reagent kits (*manufacturer Lotus NL B.V. The Hague, Netherlands*). The hypersensitive-C-reactive protein (hs-CRP) parameter was used as an inflammatory index because it constitutes the main protein of the acute phase reflecting the real inflammatory activity in humans and is a predictive factor of morbidity and poor prognosis during chronic diseases [5]. Subsequently, we determined the concentration of interleukin 6

in patients according to the 'Elabscience R' kits (*manufacturer Elabscience biotech Inc, United States*) using the ELISA Sandwich (Enzyme-Linked Immunosorbent Assay) principle. The analysis was done on an Elisa chain *(ELx50TM Automated Strip Washer brand BIOTEK)*. The ferritin described below was also used to assess inflammation in patients. Inflammation was determined from quantitative methods and defined for hs-CRP cut-off values > 6 mg/L [5]; associated with IL6 > 5 pg/mL (as defined by the test kits). From this, groups were defined as 'inflamed and non-inflamed'. Ferritin threshold values ranged from 10 to 200 ng/mL.

Assessment of the iron profile was done by determination of serum-free iron using the spectrophotometric method with the kits 'Biolabo' kits (*manufacturer BIOLABO SAS, Les Hautes Rives, 02160 Maizy, France)*. The determination of transferrin was carried out according to the Human kits (*Max-Planck-Ring 21 65205 Wiesb, German)* by immunoturbidimetric method. At the same time, the concentration of the ferritin was proceeded through the protocol of the 'CALBIOTECH' kits (*manufacturer Calbiotech Inc, El Cajon, CA 92020, United States)* whose principle is an ELISA sandwich on an Elisa ELx50TM Automated Strip Washer chain brand BIOTEK. The Total Iron Binding Capacity (TIBC) was deduced from the determination of transferrinemia by the formula: TIBC (mg/L) = transferrin (g/L) × 1395 [15]. At the same time, the Transferrin or Siderophilin Saturation Coefficient (TSC) was determined according to the formula TSC(%) = (serum iron/TIBC) × 100 [15] using Biolabo kits (*manufacturer BIOLABO SAS, Les Hautes Rives, 02160 Maizy, France)*.

2.3 | Data Management and Statistical Analysis

The data collected were saved in Microsoft Excel 2016 software. Statistical analyses of the data were carried out using the statistical software R version 4.1.1. from which logistic regression analyses according to univariate and multivariate models made it possible to identify factors associated with inflammation in patients. The Smirnov–Kolmogorov test was used to determine whether the distribution of the variables followed a normal law; thus, the presentation of quantitative variables was done in median and interquartile range while that of qualitative variables was done in frequency. The Fisher's Exact test made it possible to compare the frequencies while the Wilcoxon test allowed the comparison of the medians. The significance of the statistical analysis was set for values of $p < 0.05$.

2.4 | Ethical Considerations

This study was approved by the Centre's Regional Research Ethics Committee (AUTHORISATION N° E210/CRERSHC2021). The Bafoussam Regional Hospital and the Yaoundé Central Hospital have issued collection authorizations (No. 005/L/MINSANTE/SG/DRSPO/HRB/D) and (No. 276/21/AR/MINSANTE/SG/DHCY/CM/SM), respectively issued on January 4, 2022 and May 2021. Authorization for analysis of biological samples was obtained from the University Hospital in March 2022 (No. 74/AR/CHUY/DG/DGA/CAPRC). All the procedures performed in this study followed the ethical standards of the Declaration of Helsinki and its later amendments. Patients were informed about the purpose of the study and the benefits and risks of participating. Detailed information on the study were given to the selected potential participants and/or their legal guardians (if need be) in their first official language. For participation in research study, consent was obtained from parent or legal guardian for participants under 18 years of age. However, consent was obtained from those aged 18 or over.

3 | Results

3.1 | Distribution of Socio-Demographic and Clinical Phenotypes of Patients

During our study, 149 sickle cell patients were recorded. 50.3% (75/149) of the population was male and 49.7% (74/149) female. 10 (6.7%) of the population was on hydroxyurea intake. Table 1 shows the Distribution of socio-demographic and clinical phenotypes of all patients:

From the previously mentioned Table 1, it appears that the average BMI was 17.2 ± 3.07; 65.77% of the population was underweighted. The median age of our participants is 9 [4–13] years old. Patients had a history of infectious crises (32.2%), a history of anemic attacks (52.3%), a history of cardiovascular disease (2.01%), and a history of vasoocclusive crises (54.4%).

3.1.1 | Prevalence of Inflammation in the Population

The following Figure 1 shows inflammation in the population:

From the figure above, The prevalence of inflammation in the population was 42%.

3.2 | Distribution of Socio-Demographic and Clinical Phenotypes of Patients Depending on Inflammation

Previous transfusions were on average 1 ± 0.2 years old in participants. Table 2 presents the socio-demographic and clinical phenotypes of patients registered during the study period depending on inflammation.

It appears that inflammation does not significantly influence the clinical phenotypes of participants ($p > 0.05$).

3.3 | Distribution of the Study Population According to Blood Count Profile

3.3.1 | Pattern of Hemogram Disorders in the Population

Table 3 presents hemogram disorders of sickle cell patients during the study period.

TABLE 1 | Description of socio-demographics and clinical phenotypes of all the patients.

Variables		Effective	Frequency (%)
Sex	F	74	(49.7)
	M	75	(50.3)
BMI	Underweighted	98	(65.77)
	Normal	51	(34.23)
History of infectious crises	No	101	(67.8)
	Yes	48	(32.2)
History of anemic crises	No	71	(47.7)
	Yes	78	(52.3)
History of VOC	No	68	(45.6)
	Yes	81	(54.4)
History of CVD and stroke	No	146	(98.0)
	Yes	3	(2.01)
Hydroxyurea Intake	No	139	(93,7)
	Yes	10	(6,7)
Mean VOC/month, Med (IQR)			1.00 [0.00–2.00]
Age, Med[q1-q3]			9.00 [4.00–13.0]
BMI, Mean ± sd			17.2 ± 3.07

Abbreviations: BMI, body mass index; CVD, cardiovascular diseases; CHY, Central Hospital of Yaounde; RHB, Regional Hospital of Bafoussam; VOC, vaso-occlusive crises; age (year).

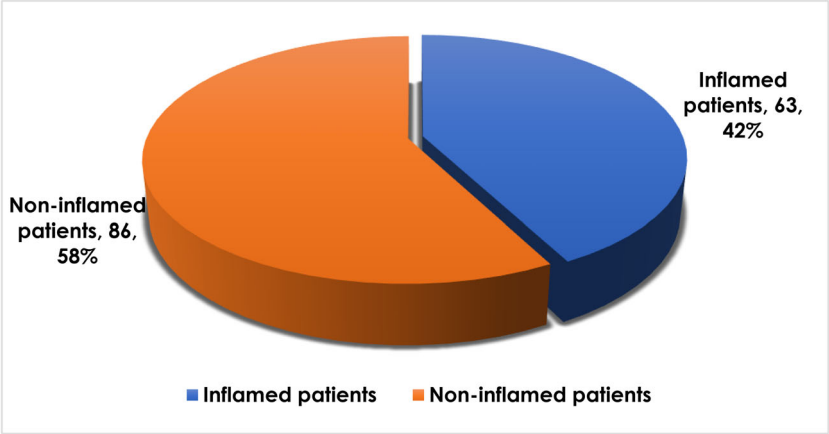

FIGURE 1 | Prevalence of inflammation in the population.

From the Table 3, 96% of the population had moderate anemia, and 7.75% had severe anemia. Moreover, 38.3% of the population has had microcytic anemia. In addition, 61.1% of the total population has experienced thrombocytosis. 66.4% of anemias were normochromic, and 33.6% were hypochromic. 95.3% of anemias were regenerative.

3.3.2 | Profile of the Complete Population Hemogram

Table 4 presents the complete blood count profile of sickle cell patients during the study period.

From Table 4, the median of the white blood cells in inflamed patients was significantly higher (17.7 G/L) compared to 15.6 G/L in non-inflamed patients ($p = 0.004$). The white blood cells, Granulocytes, Neutrophils, Eosinophils, and Basophils were significantly higher in inflamed than non-inflamed patients ($p < 0.05$). The median hemoglobin in the total population is 7.8 g/dL. Regarding platelets, the median was 460 G/mL [323–575] with 408 G/mL in inflamed sickle cell patients and 468 G/mL in non-inflamed sickle cell patients ($p = 0.005$).

3.4 | Distribution of the Study Population by Serum Parameters

Table 5 describes the serum profile in inflamed and non-inflamed sickle cell patients during the study: It appears that

TABLE 2 | Distribution of sickle cell patients from RHB and CHY by sociodemographic characteristics and clinical history of inflammation.

Variables		Inflammation: No $N = 86$ n(%)	Inflammation: Yes $N = 63$ n(%)	p-value
Sex:	F	35 (40.7)	39 (61.9)	0.017*
	M	51 (59.3)	24 (38.1)	
Age, Med [q1-q3]		8.00 [5.00-12.0]	10.0 [4.00-14.0]	0.461
BMI, Mean ± sd		17.0 ± 3.15	17.4 ± 2.96	0.482
BMI	Thin	58 (67.44)	40 (63.49)	0.864
	Normal	28 (32.56)	23 (36.51)	
History of infectious crises:	No	59 (68.6)	42 (66.7)	0.942
	Yes	27 (31.4)	21 (33.3)	
History of anemic crises:	No	43 (50.0)	28 (44.4)	0.614
	Yes	43 (50.0)	35 (55.6)	
History of VOC:	No	43 (50.0)	25 (39.7)	0.279
	Yes	43 (50.0)	38 (60.3)	
History of CVD and stroke	No	86 (100)	60 (95.2)	0.074
	Yes	0 (0.00)	3 (4.76)	
Hydroxyurea Intake	No	77 (89,5)	62 (98,4)	0,032
	Yes	9 (10,5)	1 (1,6)	
Mean VOC/month, Med (IQR)		1.00 [0.00–1.00]	1.00 [1.00–2.00]	0.072

Abbreviations: BMI, body mass index; age (year); CHY, Central Hospital of Yaounde; CVD, cardiovascular diseases; RHB, Regional Hospital of Bafoussam; VOC, vaso-occlusive crises.
*Significant difference at $p < 0.05$.

the prevalence of inflammation in the population was 42.3%. The median of ferritin in the sickle cell in patients with inflammation (669 ng/mL) was significantly higher than that of noninflammatory (426 ng/mL) patients ($p < 0.001$). The same report was found with hs-CRP and IL-6 concentrations. However, these results were contrary to those of transferrin and transferrin saturation coefficient concentrations. The median of the total iron binding capacity in the population is 3.56 mg/L. 85.2% of the population had hyperferritinemia with 96.8% in inflamed sickle cell patients and 76.7% in non-inflamed ($p = 0.001$). In addition, 84.6% of the population had an increase in the transferrin saturation coefficient with 95.2% in inflamed sickle cell patients and 76.7% in non-inflammatory sickle cell patients ($p = 0.004$). Moreover, the median IL6 was 5.01 pg/mL in patients with inflammation and 2.83 pg/mL in non-inflamed patients ($p < 0.001$). In addition, after univariate analyses, decreased transferrin, elevated TSC, and decreased TIBC were strongly associated with a risk of developing inflammation in patients with respectively (OR = 9.06; 95% CI [1.88;43.6]; $p = 0.002$), (OR = 6.06; 95% CI [1.71;21.4]; $p = 0.002$), and (OR = 3.47; 95% CI [1.46;8.2]; $p = 0.005$).

3.5 | Factors Associated With Inflammation in Sickle Cell Patients: Multivariate Analyses

Table 6 describes the factors associated with inflammation in sickle cell patients in multivariate analyses, the ferritin and IL6 elevation are associated with inflammation during major sickle cell syndromes respectively (OR = 4.96; 95% CI [1.15–36.42]; $p = 0.056$) and (OR = 6.23; 95% CI [1.43–45.96]; $p = 0.030$).

4 | Discussion

The objective of this study was to investigate the Inflammation and iron profile in children with sickle cell disease in Cameroon, identify the factors associated with inflammation, and raise the consequences for the patient including the profile of the hemogram and the iron profile. Indeed, SCD remains a monogenic disease with several unknown aspects due to its great phenotypic and genotypic variability, regulated by several known genetic factors and/or currently under study. Thus, understanding its inflammatory components on the one hand and vascular components on the other hand could contribute to providing useful information allowing a better understanding of the disease for improved patient monitoring.

Inflammation was assessed for a prevalence of 42.3% in the population. This could be explained firstly by hemolysis resulting from membrane polymerization linked to hemoglobin S. Moreover, the history of the patients of this study is characterized by a past of infectious crises, anemic crises, cardiovascular disease, and vasocooclusive crises associated with this the intrinsic factors in sickle cell disease are all factors promoting inflammation in the latter [16]. These results are similar to those of Tsihilolo et al. (2009) who reported one of two sickle cell patients in inflammation during their study in patients in the steady state [15]. Otherwise, the median hs-CRP in the population was elevated and significantly differentiated between inflamed and non-inflamed patients ($p < 0.001$) thus demonstrating a significant elevation of inflammation in the latter. These results are close to those of Cople-Rodrigues et al. (2019) and Krishnan et al. (2010) which reported medians of 5.5 mg/L and 5.6 mg/L, respectively [17, 18]. However, they are

TABLE 3 | Distribution of RHB and CHY sickle cell patients by hemogram disorders vs. inflammation.

Variables		Non-inflamed $N = 86$ n (%)	Inflamed $N = 63$ n(%)	Total $N = 149$ n(%)	p-value	OR [IC 95%]	p-value OR
RBC	Low	83 (96.5)	52 (82.5)	135 (90.6)	0.009	1.28	0.154
	High	0 (0.00)	2 (3.17)	2 (1.34)		Ref.	Ref.
	Normal	3 (3.49)	9 (14.3)	12 (8.05)		—	0.604
Reticulocyte	High	84 (97.7)	58 (92.1)	142 (95.3)	0.134	0.28 [0.05;1.47]	0.137
	Normal	2 (2.33)	5 (7.94)	7 (4.70)		Ref.	Ref.
HB	Low	84 (97.7)	58 (92.1)	142 (95.3)	0.134	0.28 [0.05;1.47]	0.137
	Normal	2 (2.33)	5 (7.94)	7 (4.70)		Ref.	Ref.
anemia	Slight	22 (55.6)	24 (38.1)	46 (32.39)	0.046*	1.78 [0.87;3.65]	0.118
	Moderate	57 (67.28)	28 (44.44)	85 (59.86)		Ref.	Ref.
	Severe	5 (5.81)	6 (9.52)	11 (7.75)		2.31 [0.65;8.27]	0.215
Hematocrit	Low	84 (97.7)	58 (92.1)	142 (95.3)	0.249	—	0.172
	High	0 (0.00)	2 (3.17)	2 (1.34)		Ref.	Ref.
	Normal	2 (2.33)	3 (4.76)	5 (3.36)		—	0.476
MCV	Microcytosis	33 (38.4)	24 (38.1)	57 (38.3)	1.000	0.99 [0.51;1.93]	0.975
	Normocytosis	53 (61.6)	39 (61.9)	92 (61.7)		Ref.	Ref.
MCH	Low	29 (33.7)	21 (33.3)	50 (33.6)	1.000	0.98 [0.49;1.96]	0.963
	Normal	57 (66.3)	42 (66.7)	99 (66.4)		Ref.	Ref.
MCHC	Hypochromia	5 (5.81)	3 (4.76)	8 (5.37)	1.000	0.81 [0.19;3.52]	0.804
	Normal	81 (94.2)	60 (95.2)	141 (94.6)		Ref.	Ref.
WBC	Leucocytosis	73 (84.9)	58 (92.1)	131 (87.9)	0.283	2.07 [0.70;6.13]	0.195
	Normal	13 (15.1)	5 (7.94)	18 (12.1)		Ref.	Ref.
Granulocytes	Low	1 (1.16)	0 (0.00)	1 (0.67)	< 0.001 *	—	0.393
	High	23 (26.7)	37 (58.7)	60 (40.3)		Ref.	Ref.
	Normal	62 (72.1)	26 (41.3)	88 (59.1)		0.26 [0.13;0.52]	< 0.001
Lymphocyte	Lymphocytosis	66 (76.7)	53 (84.1)	119 (79.9)	0.366	1.61 [0.69;3.72]	0.276
	Normal	20 (23.3)	10 (15.9)	30 (20.1)		Ref.	Ref.
Monocyte	Monocytosis	84 (97.7)	60 (95.2)	144 (96.6)	0.651	0.48 [0.08;2.94]	0.458
	Normal	2 (2.33)	3 (4.76)	5 (3.36)		Ref.	Ref.

(Continues)

TABLE 3 | (Continued)

Variables		Non-inflamed $N = 86$ n (%)	Inflamed $N = 63$ n(%)	Total $N = 149$ n(%)	p-value	OR [IC 95%]	p-value OR
Neutrophil	Neutropenia	2 (2.33)	0 (0.00)	2 (1.34)	< 0.001 *	—	0.164
	Neutrophilia	25 (29.1)	39 (61.9)	64 (43.0)		Ref.	Ref.
	Normal	59 (68.6)	24 (38.1)	83 (55.7)		0.26 [0.13;0.52]	< 0.001
Eosinophil	Eosinophilia	34 (39.5)	39 (61.9)	73 (49.0)	0.011 *	2.49 [1.28;4.84]	0.008
	Normal	52 (60.5)	24 (38.1)	76 (51.0)		Ref.	Ref.
Basophil	Basophilia	0 (0.00)	11 (17.5)	11 (7.38)	< 0.001 *	—	< 0.001
	Normal	86 (100)	52 (82.5)	138 (92.6)		Ref.	Ref.
Thrombocyte	Thrombocytopenia	2 (2.33)	3 (4.76)	5 (3.36)	0.079	1.34 [0.21;8.6]	0.789
	Thrombocytosis	59 (68.6)	32 (50.8)	91 (61.1)		0.48 [0.24;0.97]	0.041
	Normal	25 (29.1)	28 (44.4)	53 (35.6)		Ref.	Ref.

Abbreviations: MCH = mean corpuscular haemoglobin content, MCHC = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume, ref. = 0.00 [0.00], WBC = white blood cells, RBC = red blood cells.
*Significant difference at $p < 0.05$.

different from those of Tete-Benissan et al. (2011) [19] and Al-Saqladi et al. (2012) [5] which reported medians of 8.3 mg/L and 6.3 mg/L, respectively. These differences could be explained by the differences between the context of the realization of the studies. Several factors can initiate and promote inflammation in sickle patients such as infections and oxidative stress. In addition, the median IL6 was also elevated and significantly differentiated between inflamed and non-inflamed patients ($p < 0.001$); with a larger and more significant proportion in inflamed compared to non-inflamed (96.8% vs. 84.5%; $p < 0.001$); Indeed, inflammation is a factor in the increase in pro-inflammatory cytokines as also reported by several authors [16, 20, 21]. The results obtained in this study are similar to those obtained by Omena et al. in 2018 and those of Mangaonkar et al. in 2020 which reported the Interleukins 6 medians of 3.8 pg/mL and 4.5 pg/mL respectively [7, 20]. In addition, after univariate analyses, the elevation of Interleukin 6 concentration was strongly and significantly associated with a risk of developing inflammation (OR = 5.43; 95% CI [1.18;25]; $p = 0.016$).

50.3% (75/149) of the population was male and 49.7% (74/149) female. There was therefore no significant link between the type of hemoglobin and sex, this has a genetic explanation because the transmission of tare is independent of sex. These same observations were made by Dahmani et al. (2016) and Doupa et al. (2017) [11, 22]. The median age of our participants is 9 [4–13] years old corresponding to a relatively young age. This could be explained by the early mortality of sickle cell patients. Indeed, a study by Houwing et al. found that more than half of sickle cell patients die before the age of 5 in sub-Saharan Africa [23]. These sightings were also reported by Dahamani et al [22]. The population was mostly meager according to BMI. This result would suggest a significant state of malnutrition in the sickle cell population. This observation was also made by Sombodi et al [24]. Patients had a history of infectious crisis, history of anemia, history of vasooclusive crises, and history of cardiovascular disease. These described signs and symptoms are characteristic and commonly encountered in sickle cell patients. The same observations were also described in the review by Houwing et al. which provides a summary of the pathophysiology and management of sickle cell disease and encompasses the characteristics and complications of the disease; as well as that of Wonkam et al [23, 25].

Exploration of blood cells describes a high frequency (100%) of anemias with median hemoglobin as low in inflamed and non-inflamed sickle cell patients ($p = 0.008$) reflecting that inflammation does not have a considerable influence on hemoglobin in the particular context of this study. Omena et al. made a similar observation and also did not find an influence of inflammation on hemoglobin [20]. Patients had moderate anemias mainly due to the high frequency of hemolysis which is directly linked to the polymerization of the sickled red blood cell membrane [23]. Moreover, anemias were mainly normochromic, although in hypochromic cases, they were regenerative. Indeed, hyperhemolysis attacks are defined by a sudden drop in steady-state hemoglobin accompanied by increased reticulocytosis and exaggerated hyperbilirubinemia [26]. Several other studies reported cases of reticulocytosis and regenerative anemia in sickle cell patients [24, 27]. Therefore, after

TABLE 4 | Complete blood count profile.

Variables	Non-inflamed $N = 86$ a^{xxx}	Inflamed $N = 63$, a^{xxx}	Total $N = 149$ a^{xxx}	*p*-value	OR IC 95%	*p*-OR
RBC (T/L)	2.72 [2.34–3.03]	2.70 [2.51–3.09]	2.72 [2.39–3.09]	0.400	1.28 [1.80;0.91]	0.149
Reticulocytes (G/L)	355 [286–368]	345 [250–378]	350 [275–375]	0.242	1.00 [1.00;1.00]	0.060
HB (g/dL)	7.50 [7.00–8.17]	8.10 [7.55–9.30]	7.80 [7.10–8.40]	0.008	1.27 [1.59;1.02]	0.036*
HTE (%)	21.6 [19.5–23.0]	22.6 [21.0–24.9]	21.9 [20.1–24.4]	0.031*	1.05 [1.11;0.99]	0.080
MCV (fL)	80.5 [74.2–85.0]	82.0 [75.0–90.5]	81.0 [74.0–87.0]	0.127	1.02 [1.06;0.98]	0.257
MCHC (pg/c)	28.9 [25.7–31.2]	30.0 [25.4–32.5]	29.2 [25.5–31.6]	0.208	1.03 [1.11;0.95]	0.495
MCH (g/L)	35.7 [34.5–37.0]	35.8 [34.3–37.4]	35.7 [34.3–37.1]	0.963	0.98 [1.11;0.87]	0.751
WBC (G/L)	15,6 [12.5–18.2]	17.7 [13.4–23.5]	15.8 [13–20.4]	0.004*	1.00 [1.00;1.00]	0.001*
Granulocytes (G/L)	6.1 [4.7–8.4]	8.5 [5.9–10.3]	6.6 [4.9–8.9]	< 0.001*	1.00 [1.00;1.00]	< 0.001*
Lymphocytes (G/L)	6.3 [4.2–8.8]	6.1 [5.4–8.7]	6230 [4.5–8.8]	0.417	1.00 [1.00;1.00]	0.140
Monocytes (G/L)	2140 [1.5–2.8]	2.4 [1.6–3.3]	2.1 [1.5–3]	0.237	1.00 [1.00;1.00]	0.080
Neutrophils (G/L)	5.7 [4.4–7.9]	7.8 [5.5–9.6]	6.2 [4.6–8.3]	< 0.001*	1.00 [1.00;1.00]	< 0.001*
Eosinophils (/mL)	356 [275–491]	498 [346–599]	388 [288–518]	< 0.001*	1.00 [1.01;1.00]	< 0.001*
Basophils (/mL)	98.1 [75.8–135]	137 [95.5–165]	107 [79.4–143]	< 0.001*	1.02 [1.02;1.01]	< 0.001*
Thrombocytes (G/mL)	468 [386–598]	408 [268–542]	460 [323–573]	0.005*	1.00 [1.00;1.00]	0.016*

Abbreviations: a^{xxx} = median (IQR), MCH = mean corpuscular hemoglobin content, MCHC = mean corpuscular hemoglobin concentration, MCV = mean corpuscular volume, RDB = red blood cell, WBC = white blood cells.
*Significant difference at $p < 0.05$.

TABLE 5 | Distribution of sickle cell patients from RHB and CHY by serum parameters versus inflammation.

Variables		Non inflamed $N = 86$ n(%)	Inflamed $N = 63$ n(%)	Total $N = 149$ n(%)	p value	OR[IC 95%]	p value OR
Iron, a^{xxx}		1.84 [1.50–2.31]	1.91 [1.52–2.48]	1.86 [1.51–2.46]	0.616	1.06 [1.44;0.78]	0.694
Iron	Low	1 (1.16)	0 (0.00)	1 (0.67)	0.774	—	0.568
	High	74 (86.0)	57 (90.5)	131 (87.9)		Ref.	Ref.
	Normal	11 (12.8)	6 (9.52)	17 (11.4)		0.71 [0.25;2.03]	0.538
Ferritin, a^{xxx}		426 [234–660]	669 [429–835]	555 [317–749]	< 0.001*	1.00 [1.00;1.00]	< 0.001*
Ferri	High	66 (76.7)	61 (96.8)	127 (85.2)	0.001*	9.24 [2.07;41.2]	< 0.001*
	Normal	20 (23.3)	2 (3.17)	22 (14.8)		Ref.	Ref.
Tranferrin, a^{xxx}		2.89 [1.91–3.51]	2.08 [1.59–2.74]	2.55 [1.72–3.27]	0.003*	0.70 [0.92;0.53]	0.011*
TF	Low	24 (27.9)	29 (46.0)	53 (35.6)	0.007	9.06 [1.88;43.6]	0.002*
	High	15 (17.4)	2 (3.17)	17 (11.4)		Ref.	Ref.
	Normal	47 (54.7)	32 (50.8)	79 (53.0)		5.11 [1.09;23.9]	0.023
CTF, a^{xxx}		72.2 [47.7–87.8]	51.9 [39.8–68.5]	63.8 [43.0–81.7]	0.003*	0.99 [1.00;0.97]	0.011*
CST, a^{xxx}		54.7 [34.5–94.0]	65.6 [45.9–96.0]	63.6 [36.0–95.1]	0.092	1.00 [1.00;1.00]	0.614
CST	High	66 (76.7)	60 (95.2)	126 (84.6)	0.004*	6.06 [1.71;21.4]	0.002
	Normal	20 (23.3)	3 (4.76)	23 (15.4)		Ref.	Ref.
CTF	Low	21 (24.4)	22 (34.9)	43 (28.9)		3.47 [1.46;8.20]	0.005 *
	High	43 (50.0)	13 (20.6)	56 (37.6)	0.001*	Ref.	Ref.
	Normal	22 (25.6)	28 (44.4)	50 (33.6)		4.21 [1.83;9.70]	0.001*
hs-CRP, a^{xxx}		3.00 [2.00–4.50]	11.7 [8.72–22.5]	5.00 [3.00–10.0]	0.001*	—	0.940
hs-CRP	High	0 (0.00)	63 (100)	63 (42.3)	0.001*	—	—
	Normal	86 (100)	0 (0.00)	86 (57.7)		Ref.	Ref.
IL6, a^{xxx}		2.83 [1.19–4.53]	5.01 [2.87-8.59]	3.97 [1.41-6.54]	< 0.001*	1.01 [1.02;1.01]	0.002*
IL6	High	73 (84.9)	61 (96.8)	134 (89.9)	0.034*	5.43 [1.18;25.0]	0.016*
	Normal	13 (15.1)	2 (3.17)	15 (10.1)		Ref.	Ref.

Abbreviations: a^{xxx} = Med (IQR); Ferritin (ng/mL), Iron (mg/L), IL6 = Interleukine 6 (pg/mL); ref. = 0.00 [0.00], TIBC = total iron binding capacity of siderophilin (μmol/L); TSC = transferrin saturation coefficient.
*Significant difference at $p < 0.05$.

TABLE 6 | Factors associated with inflammation in patients, multivariate analyses.

Dependent: inflammation		No $N = 86$ n(%)	Yes $N = 63$ n(%)	OR (multivariable), IC 95%, *p value*
Ferritin	Normal	20 (90.9)	2 (9.1)	Ref
	High	66 (52.0)	61 (48.0)	4.96 (1.15–36.42, $p = 0.056$)
Transferrin	Normal	47 (59.5)	32 (40.5)	Ref
	High	15 (88.2)	2 (11.8)	0.67 (0.08–4.28, $p = 0.685$)
	Low	24 (45.3)	29 (54.7)	1.73 (0.41–9.02, $p = 0.476$)
TIBC	Normal	22 (44.0)	28 (56.0)	Ref
	High	43 (76.8)	13 (23.2)	0.49 (0.17–1.38, $p = 0.181$)
	Low	21 (48.8)	22 (51.2)	0.49 (0.09–2.08, $p = 0.350$)
TSC	Normal	20 (87.0)	3 (13.0)	Ref
	High	66 (52.4)	60 (47.6)	2.30 (0.50–13.36, $p = 0.308$)
IL6	Normal	13 (86.7)	2 (13.3)	Ref
	High	73 (54.5)	61 (45.5)	6.23 (1.43–45.96, $p = 0.030$*)
Hemoglobin	Normal	2 (28.6)	5 (71.4)	Ref
	Low	84 (59.2)	58 (40.8)	0.08 (0.00–0.84, $p = 0.081$)

Abbreviations: IL6 = Interleukine 6 (pg/mL); TSC = transferrin saturation coefficient (%); TIBC = total iron binding capacity of siderophilin (μmol/L).
*Significant difference at $p < 0.05$.

univariate analyses, the decrease in hemoglobin levels was associated with a risk of developing inflammation (OR = 1.27; 95% CI [1.59;2.02]; $p = 0.036$). Furthermore, the median of white blood cells was 15.8 G/L with 17.7 G/L in inflamed patients and 15.6 G/L in non-inflamed patients ($p = 0.004$); this shows the influence of inflammation on the elevation of white blood cells. Several studies also reported hyperleukocytosis in sickle cell patients [28, 29]. Sickle cell disease is indeed an inflammatory disease in which one of the markers is leukocytosis. The increase in the level and the activation of leukocytes are important mediators of inflammation in sickle cell disease causing erythroblastosis causing false hyperleukocytosis [26]. Furthermore, the median platelet in the population was also elevated (thrombocytosis) but did not differ significantly by inflammation ($p = 0.005$). Thrombocytosis in sickle cell patients is a factor promoting vasoocclusive crises [23]. This observation has been reported in several studies in sickle cell patients [11].

Exploration of the martial balance in sickle cell patients shows an elevation of ferritin with a greater elevation in inflamed patients compared with non-inflamed ones (96.8% vs. 76.7%, $p < 0.001$). In addition, the median serum iron in patients is elevated but does not show a significant difference by inflammation ($p = 0.616$). Also, a higher proportion (87.9%) of sickle cell patients present an elevation of serum iron thus reflecting a significant martial overload in the latter. Indeed, serum iron levels are increased by the action of hepcidin, a key modulator of iron metabolism following the significant inflammation observed in patients [30, 31]. Also, the elevation of ferritin would be a sign of iron overload coupled with inflammation in this present case [5]. Similar reports have been made by several authors [32]. However, inflammation did not affect the levels of iron plasma in our study. Furthermore, after univariate analyses, hyperferritinemia was strongly associated with a risk of developing inflammation in the sickle cell population (OR = 9.24; 95% CI [2.07;41.2]; $p < 0.001$). Moreover, iron overload in patients with inflammation and those not inflamed was also evaluated from a decrease in transferrin (46% vs. 27.9%, $p = 0.007$), a decrease in Total Iron Binding Capacity (34.9% vs. 24.4%, $p = 0.001$), and an increase in Transferrin Saturation Coefficient (TSC) (95.2% vs. 76.7%, $p = 0.004$). Indeed, high frequencies of hemolysis are accompanied by a reduction in serum transferrin, the capacities of which are exceeded. This has a direct consequence of an increase in the Transferrin Saturation Coefficient and a decrease in the Total Iron Binding Capacity. These observations have also been reported in several studies of sickle cell disease during inflammation [33–35]. In addition, after univariate analyses, decreased transferrin, elevated TSC, and decreased TIBC were strongly associated with a risk of developing inflammation in patients with respectively (OR = 9.06; 95% CI [1.88;43.6]; $p = 0.002$), (OR = 6.06; 95% CI [1.71;21.4]; $p = 0.002$), and (OR = 3.47; 95% CI [1.46;8.2]; $p = 0.005$). According to the multivariate model, ferritin and IL6 elevation are associated with inflammation during sickle cell disease respectively (OR = 4.96; 95% CI [1.15–36.42]; $p = 0.056$) and (OR = 6.23; 95% CI [1.43–45.96]; $p = 0.030$); thus, reflecting the important role of interleukin 6 and ferritin in inflammation. It is conceivable that our indirect index does not reflect actual hemolysis, but also that other mechanisms leading to anemia, such as iron deficiency and inflammation are involved in the pathophysiology of SCD-related vascular complications, in addition to, and independently from, hemolysis [36].

This study, despite its limitations linked to the cross-sectional nature, presents useful and necessary information for the better management of children with sickle cell disease, particularly on the need to control inflammation in the latter because it constitutes an important factor in worsening the pathophysiology of the disease, particularly on the ferric and hematological level.

5 | Conclusion

The prevalence of inflammation is high in children with sickle cell disease. During inflammation, patients are subject to a

dysregulation of iron metabolism causing its martial overload, which is a nonnegligent factor. Thus, inflammation constitutes a significant and significant factor in worsening the pathophysiology of sickle cell disease. Hence a need to control and regulate the inflammation and iron levels in sickle cell disease, which is a major factor in oxidative stress and cardiovascular disease.

Author Contributions

Romaric De Manfouo Tuono: methodology, investigation, writing – review and editing, formal analysis, writing – original draft. **Josué Louokdom Simo:** methodology, writing – review and editing, writing – original draft, formal analysis. **Prosper Cabral Biapa Nya:** writing – original draft, writing – review and editing. **Claude Tagny Tayou:** conceptualization, writing – review and editing, validation. **Constant Anatole Pieme:** conceptualization, methodology, validation, writing – review and editing.

Acknowledgments

We address our sincere thanks to the hematology laboratory staff of the '*Centre Hospitalier Universitaire de Yaoundé*', the laboratory staff of the Faculty of Medicine and Biomedical Sciences of *'Université de Yaoundé I'*, and '*Université des Montagnes*' for their help in carrying out this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Transparency Statement

The lead author Romaric De Manfouo Tuono affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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