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Are Chemokines IL-8 (CXCL8) and MCP-1 (Monocyte Chemoatractant 1) cooperating to Enhance Inflammation in Children with Sickle Cell Disease Living in Sub-Saharan Area?

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ABSTRACT

Introduction: Sickle cell disease is a genetic disease in the world with high prevalence in Sub-Saharan African countries where the prevalence varies between 20% and 30%. In Côte d'Ivoire, the prevalence is 12% and 50-75% of children with SCD do not reach their fifth birthday.

Because accurate data on cytokines are lacking in SCD in our countries, the aim of our study is to evaluate, the serum levels of MCP-1 and IL-8 in SCD African children.

Patients and methods: Patients were prospectively enrolled after an informed consent. The patients were assigned in 2 groups, steady state group and crisis group. Serums were measured by using LEGENDplexTM Human Inflammation Panel assays.

Results: 22 SCD females (52, 38%) and 20 SCD males (47.62%) were evaluated in this study. 22 (52.38%) were in crisis, while 20 (47, 62%) were in a steady state condition. This last group represented our controls.

IL-8 was higher in steady state group subjects compared to crisis subjects (1946 pg/mL ± 1384 versus 403.31 pg/mL ± 827.67, p=0.001). For MCP-1, there are no statistical differences between the two groups. (256.33 pg/mL ± 331.11 versus 261.72 pg/mL ± 324.27, p=0.9). A positive slight trend is obtained between the two chemokines in both patients group.

Conclusion: This study reveals a chronic permanent inflammatory in children with SCD living in Sub-Saharan area. A better understanding is essential to the development of a better care and new therapeutic approaches to reduce the high morbidity and mortality in our area.

Keywords: IL8, CXCL8, MCP-1, CCL2, Sickle cell disease, Children, Sub-Saharan

Abbreviations: DAMP: Damage Associated Molecular Pattern; TLR4: Toll-Like Receptor; SCD: Sickle Cell Disease; BMI: Body Mass Index; Hs CRP: High Sensitivity C Reactive Protein; MCP-1: Monocyte Chemoatractant Protein 1

INTRODUCTION

Sickle cell disease is the most prevalent genetic disease in the world with high prevalence in Sub-Saharan African countries where the prevalence varies between 20% and 30%; it is as high as 45% in some secluded areas in western Uganda [1]. The number of newborns affected by sickle cell disease is estimated at 240,000 per year or 0.3 to 25 per 1000 live births with 50-75% who do not reach their fifth birthday [2-4]. In Côte d'Ivoire, country located in West Africa, the SCD prevalence rate is 12% [5]. As such, SCD is one of the greatest public health treat of all time and represent a public health problem. This disease makes

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patients more vulnerable to infections with high mortality, organs damage and chronic anemia among children [6].

A better comprehension of the disease physiopathology in general will help to develop therapeutic approach to improve the management of the patients.

After the discovery of the abnormality in the amino sequence of the β globulin chain by Vernon Ingram, in 1956, accumulating evidence confirm a direct participation of the vascular endothelium with multifaceted cellular interactions, activating the vaso-occlusion process [7].

The permanently activated immunoinflammatory status exhibits increased levels of proinflammatory cytokines [8]. In this course, chemotactic factors have been identified. CXC and CCL chemokines represented respectively by IL-8 (CXCL-8) and MCP-1 (CCL2) exhibited respectively a chemotactic activity for neutrophils and for monocytes, macrophages and lymphocytes [9].

Early detection of inflammatory cytokines during non-crisis periods could be a useful guide for physicians towards a significant therapeutic improvement.

This study wishes to evaluate the circulating levels of monocyte chemoatractant protein-1 (MCP-1) and Interleukin 8 (IL-8) in SCD among children, their potential role in steady state and in crisis. Also, the study desires to bring its contribution to the clarification of the in vivo biological functions of chemokines in the vasoocclusion process.

PATIENTS AND METHODS

Study population

This is a prospective case control study with 42 SCD young patients from 4 to 18 years old followed at the Unité Thérapeutique transfusionnelle located in the Centre National de Transfusion sanguine in Abidjan, Côte d'Ivoire. It was conducted from October 2016 to February 2017 after approval from the national ethics board. Informed consents were obtained from the patients' parents before the patient was enrolled in this study.

Patients were divided into two groups: patient in steady state for the first group and patients admitted for crisis for the second group. The steady state was defined as a condition without crisis, illness or infection during the last three months and this group represents our control versus crisis group.

Documentation of homozygous or heterogynous sickle cell patients had been determined by hemoglobin electrophoresis on cellulose acetate strips (pH 9.2).

The vaso-occlusive crisis (VOC) patients were admitted to the unit and those who were non-symptomatic, steady state sickle cell patients coming at the unit for a routine checkup. Vaso-occlusive crisis (VOC) was defined as an episode of diffuse acute pain with infection or anemia, necessitating hospital admission and or analgesic administration. Clinical investigations were assessed by hematology specialist. Chemokines levels were compared in steady state patients and VOC patients according to sex, gender, hemoglobin profile, hematological parameters and body mass index. All subjects were coming from West African countries (Nigeria, Togo, Ghana, Mali and Guinea) but most from Côte d'Ivoire.

Chemokines assays

Blood sample were collected by veinopuncture in EDTA for the determination of the basic hematological indices and for the cytokine assay.

Blood count was obtained with an automated hematological cell counter (Sysmex XN 550 Hematology analyzer). The plasma was separated from the tube sample at 1000 g at 4°C for 10 min and stored at -30°C for chemokines assays.

Prior to use, the samples were thawed completely, mixed and centrifuged.

Plasma samples were measured by using BioLegend's LEGENDplex™ Human Inflammation Panel assays which is bead-based immunoassays, using fluorescence encoded beads. This panel allows simultaneous quantification of many human inflammatory cytokines and chemokines, but in this study we focused on chemokines MCP-1 (CCL2) and IL-8(CXCL8). The assay was performing using a filter plate in BioLegend's laboratory in San Diego, US. All samples were analyzed on the same day after being thawed.

All samples were run and analyzed on the same day after being thawed completely. A minimum of 3000 positive beads for these chemokines was acquired with a cytometer type BD FACSCalibur™. Manufactured supplied controls were used. The assay sensitivity for IL-8(CXCL8) and MCP-1 (CCL2) were respectively 1 pg/mL and 1.1 pg/mL, the intra assay precision and inter-precision ranged from 5-9% for IL-8 and MCP-1.

Data analysis was done using LEGENDplex™ Data Analysis Software when data acquisition is completed. The measurement ranges for MCP-1 and IL-8 was 0 to 10000 pg/mL

STATISTICAL ANALYSIS

All results are expressed as mean+/-SD. Data were analyzed using SPSS version 22.0 program (SPSS Inc., Chicago, Illinois, USA). Statistical results with a P value ≤ 0.05 were significant. We used the Levene Test on equal variances. For the comparison of means, we used Student test when there was an equality of variances and either Man Whitney U test or Kruskal-Wallis when there was no equality of the variances.

RESULTS

General characteristics of study subjects and circulating levels of chemokines

Demographics characteristics of the patients are summarized in **Table 1**. Data are reported as means (minimum and maximum) ± SD.

42 SCD young patients (23 HbSS homozygous type, 11 Sβ+ Thal heterozygous type and 8 βThal homozygous type) from 4-18 years, attending the Unité de Thérapeutique transfusionnelle located within the Centre National de Transfusion Sanguine in Abidjan, were enrolled in this cross-sectional study.

Table 1. Demographics characteristics

Parameters	n	Mean (min-max) ± SD
WBC (*10^9/l)	33	12663.63 (4800-22000) ± 4332.06
Hb(g/dl)	33	7.59 (5.5-9.9) ± 1.16
Neutrophils	29	5501.21 (1427.40-11346) ± 2599.46
Monocytes	29	1119.58 (421.6-3496) ± 616.30
Hemoglobin type	42	**Percentages**
AFA2	8	19.05%
SAFA2	1	2.38%
SC	1	2.38%
SFA2	11	26.19%
SSFA2	23	54.76%

22 SCD females (52, 38%) and 20 SCD males (47.62%) were evaluated in this study. 22 (52.38%) were in crisis, while 20 (47, 62%) were in a steady state condition. This last group represented our controls. The "healthy" group was constituted of 20 controls with 7 males and 13 females and mean age 12 years +/-3.8. The crisis group was constituted of 22 patients with 9 females and 13 males and the mean age 9 years +/-4.08 (**Table 2**).

Table 2. IL-8 and MCP-1 levels in age and sex matched control.

Items	Crisis SCD patients (n=22)		Steady state SCD patients (n=20)	
	Age (years)	Sex	Age (years)	Sex
	Mean=9	1.44*	mean=12	0.54*
	SD=4.81		SD=3.08	
IL-8	*Spearman=-0.199*	*p=0.61*	*Spearman=0.555*	*p=0.31*
MCP-1	*Spearman=-0.095*	*p=0.62*	*Spearman=0.606*	*p*=0.43

Circulating levels of IL-8 and MCP-1

7 SCD patients with crisis (31.81%) have IL-8 levels under 10pg/mL and among them 3 have undetectable levels.

IL-8 was higher in steady state group subjects compared to crisis subjects (1946 pg/mL ± 1384 versus 403.31 pg/mL ± 827.67, p=0.001). For MCP-1, there are no statistical differences between the two groups. (256.33 pg/mL ± 331.11 versus 261.72 pg/mL ± 324.27, p=0.9) **(Table 3)**.

When it comes to the hemoglobin type, circulating levels of Il-8 and MCP-1 are higher in AFA2 patients followed by SSFA2 and SFA2 patients **(Table 4)**.

Relationship between chemokines levels and variables

IL-8 and MCP-1 plasma levels were not correlated respectively with neutrophils count and monocytes count (r^2=0.031, r^2=0.001) **(Figures 1A and 1B)**. Relationship between the BMI (Body mass Index) and plasma levels was determined by a regression analysis. **Figure 1C** shows no - correlation of the BMI and the levels of chemokines. (r^2=0.028 for IL-8 and 0.02 for MCP-1). On the other hand, a positive slight trend is obtained between the two chemokines in both patients group (r^2=0.14). A positive correlation was found for both chemokines according to age in the steady state group (spearman 0.55 for IL-8 and 0.60 for MCP-1 **(Table 2)**.

Table 3. Comparison of circulating levels of IL-8 and MCP-1 in SCD patients.

Items	n	IL-8 (pg/ml) Mean (min-max) ± SD	P value	MCP-1(pg/ml) Mean (min-max)± SD	P Value
Age					
<10	16	433.86 (0-5475.32) ± 787.32	0.015*	305.88 (38.57-1015.13) ± 375.33	0.46**
≥ 10	26	1571.59 (0-5475.32) ± 1464.84		229.99 (33.86-1515.52) ± 291.92	
Sex					
F	22	1474.08 (3.35-5475.32) ± 1461.50	0.09**	350.73 (33.86-1515.52) ± 414.00	0.16*
M	20	768.67 (0-475.32) ± 1162.50		157.89 (3.35-5475.32) ± 128.07	
BMI					
OW	8	964.31 (0-2567.45) ± 1097.64	0.79****	437.31 (59.49-1015.52) ± 438.15	0.25***
NW	16	1278.43 (0-5475.32) ± 1627.99		265.93 (33.86-1515.52) ± 371.17	
UW	18	1098.20 (0-3638.6) ± 1268.74		157.83 (42.67-436.54) ± 121.80	
Clinical status					
Crisis	22	403.31 (0-3786.98) ± 827.67	0.001*	256.33 (33.86-1015.13) ± 331.11	0.95**
Steady state	20	1946.51 (5.37-5475.32) ± 1384.67		261.72 (38.57-1515.52) ± 324.27	
Hemoglobin type					
SSFA2	23	1161.16 (0-2567.45) ± 1077.98	0.06***	283.41 (33.86-1015.13) ± 316.41	0.47
SFA2	11	428.69 (0-3499.83) ± 1049.08		158.07 (38.57-537.95) ± 142.90	
AFA2	8	2047.60 (0-5475.32) ± 1964.94		327.06 (58.71-1515.52) ± 497.28	

Mann-Withney U test- Test de student-*** Kruskal-Wallis-Test Anova*****

DISCUSSION

The role of IL-8 and MCP-1 were evaluated in SCD patients. Of our young SCD patients, 22 (52.38%) were in crisis, and 20 (47.62%) were in a steady state status and represent our controls.

Circulating IL-8 levels were higher in steady state compared to crisis patients (1946 pg/mL ± 1384 versus 403.31 pg/mL ± 827.67, p=0.001). Among the SCD, the highest plasma levels of IL-8 were obtained with SAFA2 hemoglobin type (Sβ+Thalassemia). This may suggest that IL-8 could be used as a prescreening crisis biomarker as it is in other pathology [10] but this study cannot draw any definitive conclusion at this point.

The plasma levels of IL-8 were almost five-fold lower in patients with crisis than in controls.(p=0.001) Pathare demonstrated a progressive rise between controls, no crisis and crisis patients however no significative difference was observed [11]. Others studies show low circulating levels of IL-8 in SCD patients with crisis associated with infection and dehydratation [12,13]. In less developed countries, infections and micronutrients deficiencies especially zinc represent a significant factor of mortality and morbidity [14]. Most people living with sickle cell disease in Africa have a clinical course more severe because of infectious, and a limited management of the disease [15]. Even during steady state, levels of chemokines were increased in plasma but these levels were higher in vasoocclusive state [16-19]. To explain low IL-8 levels in some VOC patients, the authors think that genetic background involvement should play an important role in association with specific crisis inducing factors [16].

Table 4. Means levels of IL-8, MCP-1 and BMI according to the hemoglobin type.

	SSFA2			SFA2			AFA2		
	Crises (N=12)	**Steady State (N=11)**	**P**	**Crises (N=7)**	**Steady State (N=4)**	**P**	**Crises (N=2)**	**Steady State (N=6)**	**P**
IL-8	403.27 (0-2489.75) ± 688.64	1987.95 (0-2567.45) ± 770.46	0.002	163.66 (0-840.73) ± 313.15	892.49 (5.3-3499.83) ± 1738.32	0.44	1819. 3 (0-3638) ± 2572.87	2123.71 (130.10-5475.32) ± 2013.36	0.73
MCP-1	323.81 (33.86-147.79) ± 424.09	239.34 (59.49-436.54) ± 135.36	0.28	197. 19 (51.8-537.95) ± 168.87	89.62 (38.57-113.11) ± 34.85	0.13	242.17(71.21-413.14) ± 241.78	355.36 (58.71-1515.32) ± 575.04	0.73
BMI	17.73 (11.83-23.66) ± 4.4	17.29 (13.71-23.53) ± 4.19	0.88	17. 96 (13.14-21.89) ± 2.76	16. 79 (10.32-21.87) ± 4.95	0.8	12. 81	16.75 (12.39-19.89) ± 3.32	0.3

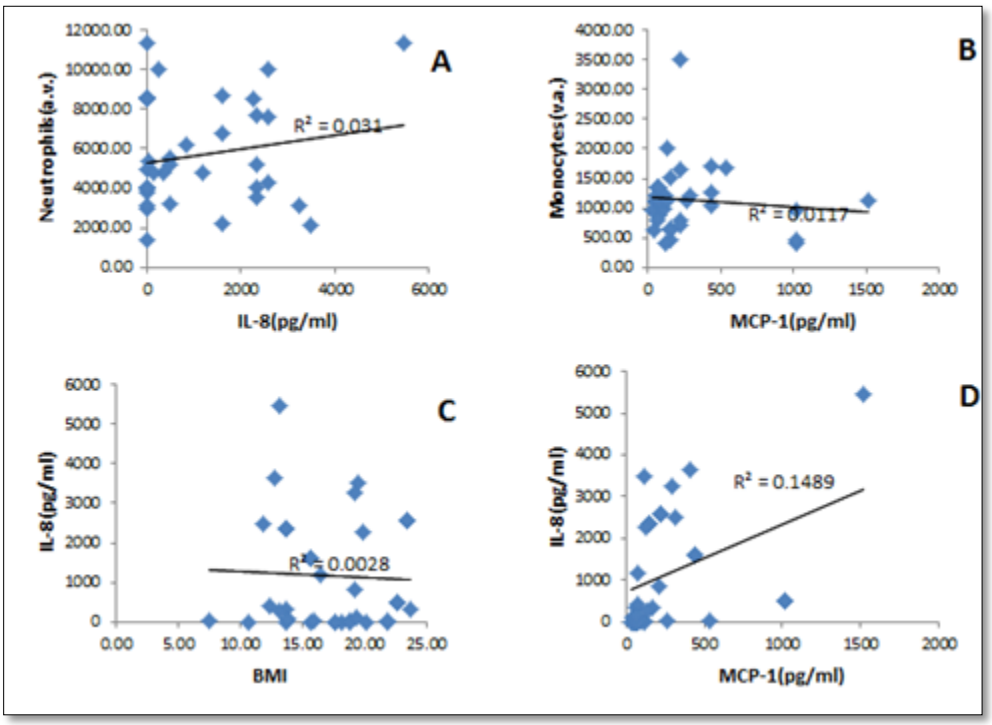

Figure 1. Linear regression analysis for IL-8 and MCP-1 levels (pg/ml) and neutrophils, monocytes counts and BMI. A) spearman rank correlation: 0.12; B) Spearman rank correlation: 0.007; C) Spearman rank correlation: -0.022; D) Spearman rank correlation: 0.66.

Recently, a novel theory that the intestinal microbiota should trigger VOC has been proposed. Depletion in intestinal microbiota may reduce the number of circulating aged neutrophiles and improves pathogenesis and inflammation [20].

Much evidence indicates that inflammation is a key factor in SCD physiopathology [21]. Hemolysis releases erythroid DAMP molecules which can trigger a sterile inflammatory reaction involving TLR4 activation and stimulates neutrophils to release NETs and activate immune response [22].

The production of IL8 during acute chest syndrome may increase sickle erythrocytes adhesion, neutrophil activation leading to organ damage and lung fibrosis, increasing risks of mortality and stroke in SCD [23].

According to Goncalves [16], the deep knowledge of kinetic chemokines is important to understand how the levels increase or decrease before or after the crisis.

Another aspect should be taken in account. A conflict may exist between the level in the blood and the level in the tissue where the inflammatory response is present and a small level in blood may result in higher level locally. Tam et al. [24] hypothesis that the systemic may underestimate the concentrations at a local level. Therefore our results should also be interpreted with finesse and further studies should take in consideration both local and systemic aspects.

Several vasodilating mediators and leukocyte chemotactic factors are produced at the site of injury and these mediators synergistically induce vasodilatation and recruitment of leukocytes, thereby establishing inflammatory reactions [21,25]. Neutrophil infiltration into inflammatory sites is one of the hallmarks of acute inflammation. High leucocytes count is present in chronic inflammation because of extravasation of plasma [26].

In our patients, high leucocytes and neutrophils count in particular was detected and showed increased circulating levels. White blood cells (WBC) mean and neutrophils mean count did not differ statistically between those with lower levels (<15 pg/mL) of IL-8 (WBC: 12833+/-4599, neutrophils: 4806.31+/-3296.56) and high levels (WBC: 13109.37+/-4370.30, neutrophils: 6052.32+/-2520). WBC count did not differ according to the type of hemoglobin.

Leukocytosis is almost constant in SCD patients even in steady state patients and predicts for stroke, acute chest syndrome and overall mortality.

High leucocytes count is a factor of morbidity [7,27] because there are present in post-capillary veinules and slow down the circulation initiating vaso-occlusive crisis (4).

We did not test CRP in our patients but many datas are in favor of association of VOC frequency and increased level of Hs CRP support the need of testing CRP in VOC follow up [28]. Baseline hemolytic activity is correlated with inflammation and this is materialized with an inverse correlation between Hb level and Hs CRP level [8].

We also investigated the association between levels of selected chemokines in our study and Body Mass Index (BMI). Patients were classified in three groups: patients with overweight (19.05%), patients with underweight (42.86%) and patients with normal BMI (38.10%). Chemokines were not significantly different between normal weight, overweight and underweight and the levels of cytokines IL-8 and MCP-1 were not correlated with BMI.

In contrast [29,30], IL-8 was lower in overweight group compared to underweight and normal BMI. Obesity does not exist among our sickle cell African patients.

Our study has some limits. Due to lack of financial aspect, small number of patients was enrolled. The challenge in the future for a better care of SCD patients in crisis is important but before crisis is better. This information's need to be checked in larger cohorts to confirm our findings.

CONCLUSION

The physiological role of cytokines in SCD is well established. However, poor data are available for children born with SCD in Sub-Saharan where the disease is endemic and environmental aspects different. Our data demonstrated that the levels of IL-8 and MCP-1 were significantly higher in steady state subjects compared with those in crisis and IL-8 was positively related to the levels of MCP-1 in subjects, supporting the idea that these chemokines are both implicated in inflammatory process in SCD.

Further investigations for a better understanding of this disease need to be conducted monitoring the levels of chemokines in a more consistent group. These biomarkers may be helpful for phenotyping SCD patients for both research and therapy.

NO CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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