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Determination of Plasma Levels of Inflammatory Cytokines in Sickle Cell Patients at the Central Hospital of Yaoundé-Cameroon

Manuella Kouemo Tchokote[1], Chris Mbianda Nana[2], Bernard Chemegni Chetcha[1], Rosette Megnekou[2], Benderli Christine Nana[2], Reine Medouen Ndeumou[2], Amaelle Goretti Mvong[1,3], Claude Tayou Tagny[1*]

[1]Department of Microbiology, Parasitology, Hematology, Infectious Diseases and Public Health, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon
[2]Department of Animal Biology and Physiology, Faculty of Sciences, University of Yaoundé I, Yaoundé, Cameroon
[3]Department of Research, Elisabeth Glaser Pediatric AIDS Foundation, Douala, Cameroon
Email: *tayouclaude@yahoo.fr

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Abstract

Background: Sickle cell disease is the most common genetic disorder worldwide and the most prevalent in the African region. It has been recognized as a public health priority by UNESCO in 2005, the African Union in 2005, WHO in 2006, and the United Nations in 2008. The objective of our work was to determine the level of inflammatory cytokines in sickle cell patients. **Methodology:** This cross-sectional, descriptive, and prospective study was conducted over 18 months (February 2020 to August 2021) and included 80 sickle cell patients. We performed CBC, blood smear, CRP, and cytokine assays and looked for factors associated with elevated cytokines in these patients. **Results:** The study showed that 56% of participants were women, and the most represented age group was 15 to 30 years (82.5%). The main reason for hospitalization was vaso-occlusive crises (VOC) with a prevalence of 76%. The prevalence of blood transfusions ranged from 2 to 4 per year, and these patients had hemoglobin levels between 6 and 11 g/dL. Only the cytokine IL-6 was significantly higher in patients experiencing vaso-occlusive crises compared to those in a stable state. No significant correlation was observed between plasma concentrations of different cytokines and the number of attacks. **Conclusion:** IL-6 titration may have prognostic and therapeutic value in managing sickle cell disease.

Keywords

Sickle Cell Disease, Vaso-Occlusive Crises, Transfusion, Cytokines

1. Introduction

Sickle cell disease, also known as sickle cell anemia, is a common hereditary disease caused by a hemoglobin defect. It is the most common genetic disease in the WHO African region [1]. In Central Africa and Cameroon, the mortality rate for sickle cell patients is 50 to 75% before the age of 5. Children under 5, adolescent girls, and pregnant women are most affected [1]. Additionally, 7% of the world's population carries an abnormal globin gene, and in some parts of the world, up to 1% of newborns are affected by a hemoglobin disorder. Of the more than 1600 hemoglobin mutants known, over 1200 are responsible for the synthesis of an abnormal variant [2]. This disease includes broad categories of clinical manifestations such as hemolytic anemia, painful attacks, and increased susceptibility to infections. To prevent or remedy these situations, several methods can be used, such as antibiotics, a healthy lifestyle, or blood transfusion. Blood transfusion is an important tool in managing sickle cell disease [2]. It involves transfusing the patient with blood from a compatible healthy donor, restoring an acceptable level in cases of severe anemia. Despite its advantages, red blood cell transfusion leads to alloimmunization in about 20% - 50% of patients with sickle cell disease [3]. Alloimmunization results from antigen presentation, positively influenced by pro-inflammatory cytokines and negatively by regulatory T cells [4]. Concentrations of the cytokines IL-6 and TNF-α were significantly elevated in people with sickle cell disease presenting with a vaso-occlusive crisis [5]. Alloantibodies can cause delayed hemolytic transfusion reactions, which in sickle cell patients can trigger hyperhemolysis. Many studies have reported that alloimmunization is associated with morbidity and mortality in chronically transfused sickle cell patients. However, there are a few additional or alternative treatment options besides transfusion [6]. Genetic and acquired factors related to sickle cell patients likely influence the alloimmunization process. Previous studies have shown that several factors may be involved in alloimmunization, such as genetic factors, subset phenotypes of regulatory immune cells, immune activation status, and functional characteristics of immune cells [7]. Some studies in sickle cell and chronically transfused patients have shown reduced suppressive function of peripheral regulatory T cells (Treg) and impaired Th responses with higher circulating IFN-α but low concentrations of the anti-inflammatory cytokine IL-10 [8]. Given the lack of information on this issue in our country, we felt it was important to carry out this study on the determination of plasma levels of inflammatory cytokines in sickle cell patients at the Yaoundé Central Hospital, with the ultimate aim of contributing to the improvement of management and quality of life for these patients.

2. Methodology

2.1. Study Design

This prospective, cross-sectional, descriptive study took place from February 2020 to August 2021 in the hematology department of the Yaoundé Central Hospital,

involving sickle cell patients of all ages who voluntarily agreed to participate. Patients with diseases such as HIV, hepatitis, diabetes, hypertension, and other inflammatory diseases were excluded. Ethical clearance was obtained from the ethics committee of the Faculty of Medicine and Biomedical Sciences at the University of Yaoundé I. Administrative authorizations were obtained from the heads of the target health facilities. Verbal and written informed consent was obtained from the patients, and information was collected on dedicated anonymous forms. Sampling was consecutive over three months, from April 26, 2021, to July 31, 2021.

2.2. Sample Collection

The blood samples were collected in two tubes (EDTA and dry), then aliquoted into two separate tubes (serum and plasma) and stored at +20˚C. The hematological and immunological analyses were carried out at the Yaounde University Hospital (CHUY) and the Nkolbisson Biotechnology Centre (CBT), respectively.

➢ **Determination of cytokines IL-1 beta/IL-1F2, IL-4, IL-6, IL-8/CXCL8, IL-10, IFN-gamma, and TNF-alpha using Luminex MAGpix** The plasma concentrations of various cytokines were determined using a multianalyte platform assay, following the experimental protocol provided by the assay kit (Human Premixed Multi-analyte Kit, L140288, R&D Systems, Inc., Minneapolis, MN, USA). The principle involves the simultaneous detection of several antigens using antibodies pre-coupled to magnetic beads with coded fluorescence spectra and detection antibodies bound to the fluorochrome phycoerythrin (PE) via the streptavidin-biotin system. The resulting complex is read using the Luminex MAGpix. The Median Fluorescence Intensities (MFIs) obtained, plotted against the standard cytokine concentrations, allowed the interpolation of plasma concentrations in pg/mL of the different cytokines for each sample.

➢ **TGF-beta 1 assay by ELISA**

The assay kit was a DuoSet ELISA (human TGF-beta 1 & Ancillary Reagent kit 1) for determining the cytokine TGF-β1. The principle is based on the SANDWICH enzyme-linked immunosorbent assay, which involves using a capture antibody attached to the bottom of the wells and a detection antibody that binds streptavidin-HRP and the substrate. Optical densities were determined using the SPECTRAmax PLUS 384 Microplate Spectrophotometer ELISA reader and SOFTmax PRO software.

The duplicate readings for each standard were averaged, and the average optical density of the zero standard was subtracted. A standard curve was then created using software capable of generating a four-parameter logistic curve fit.

2.3. Statistical Analysis

GraphPad Prism version 6.0 and Microsoft Excel were used to analyze the data. The Spearman Rank Order Correlation test was used to determine the correlations between the different parameters. The difference was significant at a p-value <

0.05.

2.4. Variables Studied

- Sociodemographic factors: age and sex.
- Clinical status: number of vaso-occlusive crises and blood transfusions per year.
- Biological status: hemoglobin level and CRP.
- Biological status: hemoglobin, CRP, and inflammatory cytokines IL-1β, IL-4, IL-6, IL-8, IL-10, INF-γ, TNF-α, and TGF-β1.

3. Results

3.1. Sociodemographic Profile of Sickle Cell Patients

Our study population was predominantly female, with 45 girls and 35 boys, resulting in a sex ratio of 1.28 (Figure 1). The age of these patients ranged from 12 to 41 years, with a median age of 22 years. The most represented age group was 15 to 30 years (66 patients), accounting for 82.5% of the participants (Figure 2).

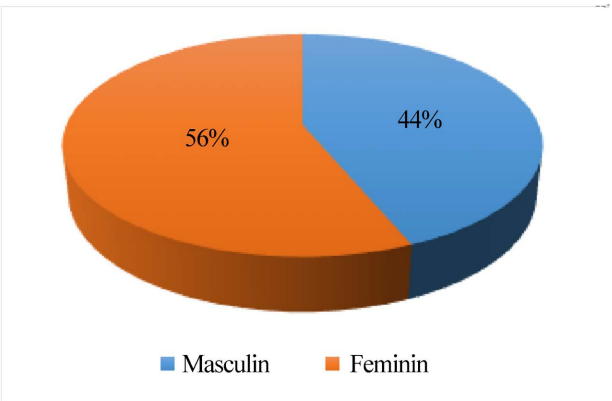

Figure 1. Distribution of the population by gender.

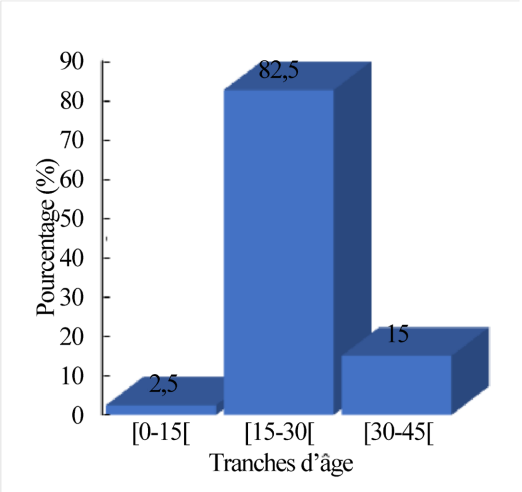

Figure 2. Distribution of the population by age.

3.2. Clinical and Biological Profile in Sickle Cell Patients

The recruited sickle cell patients had a median of three severe seizures requiring hospitalization per year. At the time of recruitment, 19 patients (24%) had a seizure, and 61 patients (76%) were stable. Annually, about 20 patients (25.6%) experienced only one vaso-occlusive crisis, while more than half, 43 patients (53.75%), experienced between 3 and 5 crises, as shown in Figure 3. The proportion of patients experiencing more than six seizures per year is very low, at 2.5% (Figure 3). The median number of blood transfusions per year among the patients was two. Ten patients (12.5%) did not receive a blood transfusion. The proportion receiving between two and four transfusions per year was 46.8% (Figure 4). Thirty-three patients (41.25%) had a hemoglobin level between 6 and 8 g/dL, and one patient (1.25%) had severe anemia (hemoglobin level < 4 g/dL) (Figure 5). Nineteen sickle cell patients had a CRP above 192 mg/L (Figure 6).

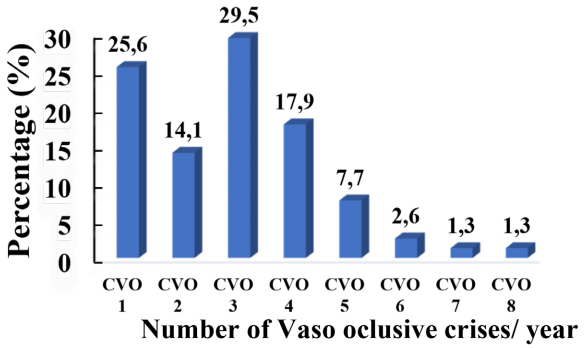

Figure 3. Distribution of the population according to the number of CVOs.

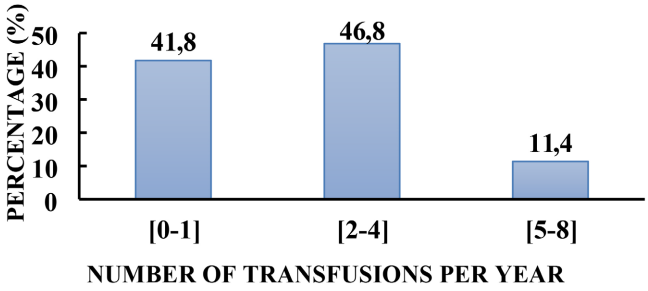

Figure 4. Distribution of the population according to the number of blood transfusions.

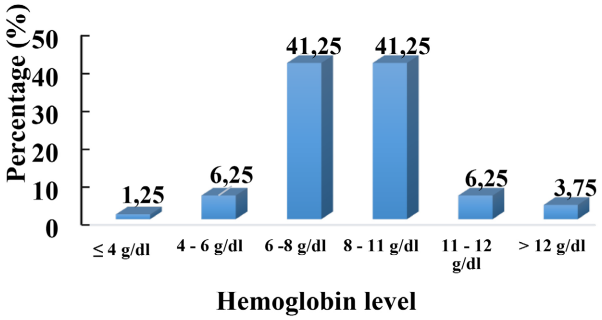

Figure 5. Distribution of the population according to hemoglobin level.

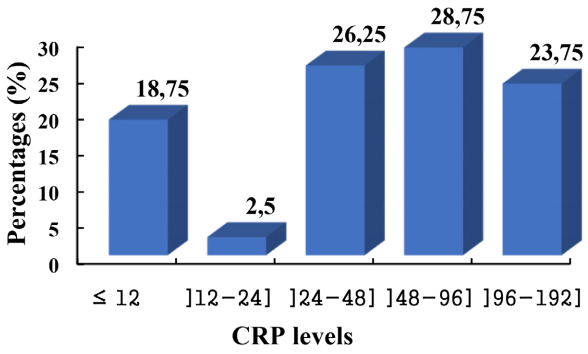

Figure 6. Distribution of the population according to CRP rate.

3.3. Plasma Concentrations of the Inflammatory Cytokines IL-1β, IL-4, IL6, IL-8, IL-10, INF-γ, TNF-α and TGF-β in Drepania Patients

The median concentrations of different cytokines were determined in sickle cell patients. The cytokines IL-10, IL-1β, and IL-4 had a median concentration of zero pg/mL, while TNFα was 4.504 pg/mL, IL-6 was 1.63 pg/mL, IL-8 was 10.03 pg/mL, INF-gamma was 4.815 pg/mL, and TGF-β1 was 9.134 pg/mL (Table 1).

Table 1. Plasma concentration of cytokines in sickle cell patients.

Variable	TNF-α	IL-6	IL-8	IL-10	IL-1β	IFN-γ	IL-4	TGF-β1
Minimum	0	0	0.07724	0	0	0	0	0
25% Percentile	4.504	0	6.589	0	0	0	0	0.1503
Median	4.504	1.63	10.03	0	0	4.815	0	9.134
75% Percentile	7.872	4.933	18.43	5.38	14.64	4.815	0	21.59
Maximum	24.43	115.5	91.83	259.9	80.19	422	0	84.92

3.4. Factors Associated with the Elevation of These Cytokines in Sickle Cell Patients

Table 2. Plasma levels of cytokines as a function of age, number of blood transfusions per year, number of crises per year, hemoglobin level, and CRP level.

Variables	Age		Number of transfusions/year		Number of crises/year		Hemoglobin level g/dL		CRP (mg/L)	
	rs	p	Rs	p	rs	p	rs	P	rs	p
TNF-α	0.121	0.283	0.088	0.437	0.045	0.692	−0.263	0.018	0.362	0.001
IL-6	0.193	0.086	0.118	0.298	0.107	0.349	−0.227	0.042	0.419	0.0001
IL-8	−0.090	0.428	−0.066	0.560	0.025	0.828	0.022	0.846	0.214	0.056
IL-10	0.0330	0.772	−0.048	0.672	−0.044	0.695	0.0484	0.670	0.153	0.176
IL-1β	0.081	0.475	−0.030	0.790	−0.063	0.578	0.186	0.098	−0.056	0.616
IFN-γ	−0.131	0.246	0.027	0.807	0.204	0.070	−0.053	0.640	0.046	0.685
TGF-β1	0.259	0.043	0.019	0.881	−0.150	0.252	−0.217	0.093	0.017	0.897

The results show that the plasma level of the cytokine IL-6 was significantly higher in sickle cell patients in vaso-occlusive crisis (VOC) compared to those in steady state (DAE) (p = 0.02) (Figure 7). The correlation results indicate that the cytokines TNF-α and IL-6 correlated negatively [(rs = −0.263; p = 0.018); (rs = −0.227; p = 0.042) respectively] and positively with CRP levels [(rs = 0.362; p = 0.001); (rs = 0.419; p = 0.0001) respectively]. No significant correlation was observed between the differences in cytokine concentrations of TNF-alpha, IL-6, IL-8, IL-10, IL-1β, IFN-gamma, TGF-β1 and age, number of transfusions, number of attacks, hemoglobin level, and CRP (Table 2).

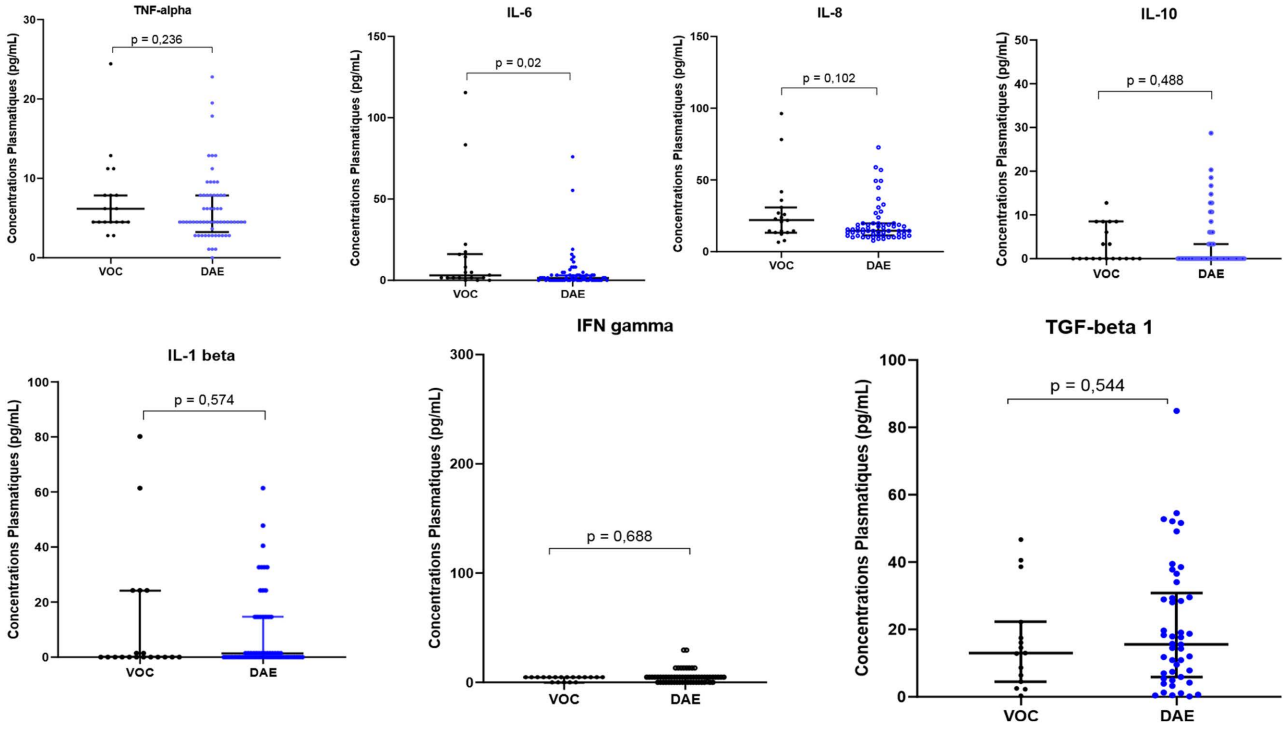

Figure 7. Plasma cytokine levels in sickle cell patients during vaso-occlusive crisis (VOC) and in steady state (DAE).

4. Discussion

In our study, women are the majority, with a sex ratio of 1.28 male/female (45 women to 35 men). This predominance of women can be explained by the higher number of women in the Cameroonian population, with a similar sex ratio of 1.0009 men/women [9]. The most represented age group was sickle cell patients aged 15 to 30, with a median age of 22 years. These values are higher than those obtained by Zohreh *et al.* in Washington in 2014, where a study on high multicytokine titer in sickle cell alloimmunization reported a median age of 14 years [4]. In our study, we found a hospital prevalence of CVO of 76%, which is the leading cause of hospitalization in sickle cell patients, with a median of three attacks per year. Our results are similar to those obtained by Diagne *et al.* in Senegal in 2000 [10], who found that CVO was the primary reason for hospitalization with a prevalence of 67%. Additionally, Milica Brozovic *et al.* in England in 1987 [11]

and Ouakasse in Morocco in 2015 [12] reported a higher prevalence of 80%. Hemoglobin level is a determining factor in sickle cell disease. Our results show that the majority of patients had hemoglobin levels between 6 and 11 g/dL, suggesting these patients are anemic. Our results are similar to those of Shongo *et al.* 2015 [13]. The abnormal shape of hemoglobin causes red blood cells to become deformed, fragile, and rigid. These abnormalities lead to anemia, painful vaso-occlusive crises, and an increased risk of infections. In the present study, results showed that the cytokine IL-6 was significantly higher in sickle cell patients in VOC crisis compared to those in a steady state. For other inflammatory cytokines, plasma concentrations were higher in VOC patients than in steady-state patients, although not significantly. These findings are similar to those of Sarray *et al.*, 2015 [5]. This suggests that cytokine IL-6 is involved in the severity of the disease. IL-6 is a multifunctional and pleiotropic cytokine produced by immune cells (macrophages, neutrophils) that plays a central role in host defense mechanisms. Elevated circulating levels of IL-6 can negatively impact humoral and cell-mediated immune functions in sickle cell patients, increasing morbidity risk. Furthermore, low plasma concentrations of anti-inflammatory cytokines may be due to low levels of inflammatory cytokines that have not reached a threshold to trigger increased concentrations of anti-inflammatory cytokines.

5. Conclusion

Our study population was predominantly female, with a sex ratio of 1.28. The most represented age group was 15 to 30 years, with a median age of 22. The main reason for hospitalization of sickle cell patients was CVO, with a hospital prevalence of 76%. The cytokines IL-10, IL-1β, and IL-4 had a mean plasma level of 0 pg/mL, while the cytokines TNFα, IL-6, IL-8, INF-gamma, and TGF-β1 had levels of 4.504 pg/mL, 1.63 pg/mL, 10.03 pg/mL, 4.815 pg/mL, and 9.134 pg/mL, respectively. Plasma concentrations of pro-inflammatory cytokines are higher in CVO patients with sickle cell disease, although not significantly. IL-6 may be of prognostic and therapeutic interest in managing sickle cell disease.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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