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**Original Research Article**

# Some markers of inflammation in patients with sickle cell disease at Zou-Collines departmental hospital in Benin

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## ABSTRACT

**Background:** Sickle cell disease was a genetic pathology of the red blood cell, which caused systemic functional and tissue damage. The present work aimed to study some biomarkers of inflammation and renal function in sickle cell patients.
**Methods:** The biochemical and inflammatory markers were assayed with a spectrophotometer and the hemogram was performed on a hematology analyzer in SS homozygous sickle cell subjects and controls with AA phenotypes.
**Results:** The male sex (63.49%) and the age group 5-18 years (58.73%) were predominant among the SS subjects of the study population. Blood urea and serum creatinine were significantly higher in SS, suggesting impaired renal function. Serum uric acid was significantly lower in SS. Aspartate transaminase (AST) was significantly higher in SS and alanine transaminase (ALT) did not change significantly, indicating significant cytolysis. Mean hemoglobin level was significantly lower in SS, signifying more hemolysis. The mean number of blood leukocytes, neutrophils, eosinophilia, lymphocytes and monocytes were significantly higher in SS. There was the same for C-reactive protein, the concentration of which was very high (96 mg/l) in 34.92% of sickle cell patients against 1.75% in AA, indicating an inflammatory state in sickle cell disease. In contrast, the mean number of blood platelets was significantly lower in SS.
**Conclusions:** During sickle cell disease, there were inflammation, cytolysis and disturbance of renal function as well as anemia. The early evaluation of these biological markers may prevent complications and crises of sickle cell disease.

**Keywords:** Sickle cell disease, Kidneys, Inflammation, Biomarkers, Benin

## INTRODUCTION

Sickle cell disease and β-thalassemia were the two most prevalent autosomal recessive hereditary hemoglobinopathies in the world.[1,2] Among the congenital hemoglobinopathies, sickle cell disease remained the most frequent and the most serious.[3] It resulted from a substitution of a pair of nucleotides which led to a propensity for the polymerization of hemoglobin and the sickling of red blood cells.[4] Renal involvement in major sickle cell syndrome was frequent and early.[5] Despite the high frequency of homozygous sickle cell disease in the different regions of black Africa, few studies were

available on sickle cell nephropathy which naturally progresses to chronic renal failure. Better management of patients, in particular homozygotes, made it possible to significantly improve their prognosis and to go beyond the fourth decade. Their evolution, however, in the long term remained complicated by organ failure.[3,6] Although sickle cell disease was a chronic disease, it could be potentially serious by the occurrence of acute complications associating three main categories of clinical manifestations which could be intertwined with each other, with a great variability of expression depending on the individual. These included ischemic, anemic and infectious complications.[7] Recent data indicated a direct involvement of the vascular endothelium, multiple cell interactions and cell activation processes, involving inflammatory mechanisms, in the initiation and propagation of vaso-occlusion.[8]

Sickle cell anemia was associated with a chronic pro-inflammatory state maintained by the frequency of severe recurrent infections and subsequent vaso-occlusive complications with leukocyte adhesion to the endothelium and increased plasma levels of inflammatory cytokines.[9-11] The factors involved in the liver damage of a patient with sickle cell disease were often multiple and may involve: vaso-occlusion of the hepatic sinusoidal capillaries, lithiasis, iron overload, chronic viral hepatitis and all the others known liver disease factors. The review of the literature on the subject made it possible to isolate a certain number of manifestations, secondary to chronic hemolysis such as lithiasis, or vaso-occlusion and intra-hepatic vaso-occlusive crises.[12]

The aim of the study was to highlight some inflammatory biomarkers that will prevent complications in order to increase the life expectancy of sickle cell patients in Benin.

## METHODS

### *Biological material and sampling*

An analytical study was conducted during the period from June 2020 to December 2020 in sickle cell disease management service of the departmental hospital center Zou/Collines in Benin. Intentional sampling was performed in all homozygous AA (control) or homozygous SS sickle cell patients admitted to the Zou/Collines departmental hospital during the study period and who gave their consent to participate in the study. Blood samples were collected from patients in tubes adapted for analysis after their consent or that of their parents for patients under 18 years of age.

All patients who met the inclusion criteria during the study period from June 2020 to December 2020 constituted the sample size since this was a study performed with purposive sampling technique. 63 SS sickle cell patients and 57 AA controls participated in the study.

The exclusion criteria for the study were the non-consent of patients or their parents (for minors), patients with renal and hepatic pathologies, patients who were under treatment for these pathologies or on anti-inflammatory drugs.

### *Ethics statement*

The study was approved by the national research ethic review boards of Benin.

Blood samples from SS homozygous patients and AA controls were taken after the consent of the latter or their parents.

### *Blood tests*

We then performed blood tests on these samples. Regarding the hematological parameters, the hemogram was performed. Then biochemical parameters such as creatinemia, uremia, uricemia, transaminases and C-reactive protein were performed for all the subjects of our study.

### *Statistical analysis*

The results were expressed as a mean plus or minus standard error on the mean (mean±SEM). The parameters of patients with hemoglobin SS were compared to those of patients with hemoglobin AA using the student test. The graphs were built with Graghpad software. The significance level was set at 5%.

## RESULTS

### *Socio-demographic characteristics of the study population*

Table 1 presents the age groups and sex of the patients in the study.

The average age of our study population was 13 years in SS subjects and 21 years in AA controls. In the group of SS homozygotes, the most represented age group in our study was that of 5 to 18 years with a frequency of 58.73%. In the control group, the age group from 18 to 52 was more represented (54.39%). The male sex was predominant in our study population with a sex ratio of 1.73 and 1.11 respectively in SS subjects and AA control subjects.

### *Exploration of renal function*

Figure 1 showed the results of serum uremia and serum creatinine.

The mean uremia was 0.2±0.01 g/l in AA patients and 0.3±0.01 g/l in SS patients. It was significantly higher in the SS ($p<0.05$).

Mean serum creatinine was 8±0.3 mg/l in AA patients and 10±0.3 mg/l in SS patients. It was significantly higher in the SS ($p<0.05$). The increase in these two parameters indicated renal suffering.

**Table 1: Ages and sex of subjects.**

| Variables | SS subjects | | AA subjects | |
|---|---|---|---|---|
| | N | % | N | % |
| **Age groups** | | | | |
| 0-5 | 10 | 15.87 | 05 | 8.77 |
| 5-18 | 37 | 58.73 | 21 | 36.84 |
| 18-52 | 16 | 25.4 | 31 | 54.39 |
| Total | 63 | 100 | 57 | 100 |
| **Sex** | | | | |
| Male | 40 | 63.49 | 30 | 52.63 |
| Female | 23 | 36.51 | 27 | 47.37 |
| **Sex ratio (M/F)** | 1, 73 | | 1, 11 | |

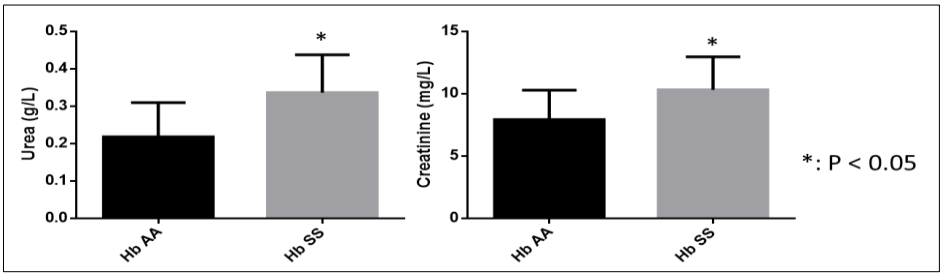


**Figure 1: Comparison of uremia and serum creatinine between AA and SS.**

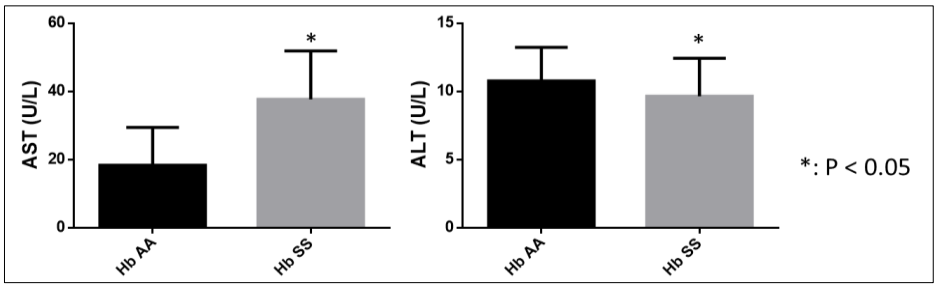


**Figure 2: Comparison of AST and ALT transaminases between AA and SS.**

***Exploration of hepatic function***

Figure 2 showed the results of AST and ALT transaminases.

The mean AST transaminase was 18±2 U/l in AA patients and 38±2 U/l in SS patients. It was significantly higher in the SS ($p<0.05$), indicating increased cytolysis. That of ALT transaminase was 11±1 U/l in AA patients and 10±1 U/l in SS patients. It was significantly lower in the SS ($p<0.05$).

***Exploration of inflammatory parameters***

Figure 3 and Tables 2 and 3 showed the results of uric acid, blood count and C-reactive protein.

Mean serum uric acid was 58±2 mg/l in AA patients and 49±2 mg/l in SS patients. It was significantly lower in the SS ($p<0.05$), suggesting little degradation of nucleic acids in sickle cell patients.

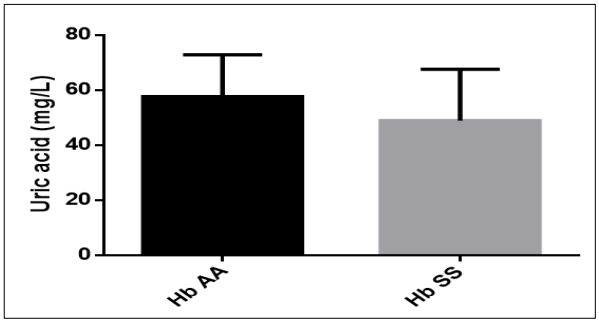


**Figure 3: Uric acid level AA and SS subjects.**

**Table 2: Blood count of AA and SS subjects.**

| Parameters (g/l) | SS patients | AA patients | P value |
|---|---|---|---|
| **Hemoglobin level (g/dl)** | 7.6±2 | 11.3±0.2 | <0.05 |
| **Leukocyte count** | 10.7±2 | 6.5±0.2 | <0.05 |
| **Neutrophils count** | 6.5±1 | 3.9±0.1 | <0.05 |
| **Eosinophil count** | 0.15±0.01 | 0.13±0.01 | <0.05 |
| **Lymphocyte count** | 3.3±0.07 | 2.02±0.07 | <0.05 |
| **Monocytes count** | 0.67±0.2 | 0.41±0.01 | <0.05 |
| **Platelet count** | 137±4 | 190±4 | <0.05 |

The mean hemoglobin level was 11.3±0.2 g/dl in AA patients and 7.6±2 g/dl in SS patients. It was significantly lower in SS (p<0.05), what explained the frequent anemia in sickle cell disease.

The mean number of blood leukocytes was 6.5±0.2 g/l in AA patients and 10.7±2 g/l in SS patients. It was significantly higher in the SS (p<0.05).

The mean blood neutrophil count was 3.9±0.1 g/l in AA patients and 6.5±1 g/l in SS patients. It was significantly higher in the SS (p<0.05).

The mean blood eosinophil count was 0.13±0.01 g/l in AA patients and 0.15±0.01 g/l in SS patients. It was significantly higher in the SS (p<0.05).

The mean number of blood lymphocytes was 2.02±0.07 g/l in AA patients and 3.3±0.07 g/l in SS patients. It was significantly higher in the SS (p<0.05).

The mean number of blood monocytes was 0.41±0.01 g/l in AA patients and 0.67±0.2 g/l in SS patients. It was significantly higher in SS (p<0.05). The increase in the number of white blood cells indicated inflammation in sickle cell disease.

The mean platelet count was 190±4 g/l in AA patients and 137±4 g/l in SS patients. It was significantly lower in the SS (p<0.05).

**Table 3: Distribution of subjects according to CRP.**

| CRP (mg/l) | <6 | 6-48 | ≥96 | Total |
|---|---|---|---|---|
| **SS subjects** | | | | |
| Number | 22 | 19 | 22 | 63 |
| Frequency | 35 | 30 | 35 | 100 |
| **AA subjects** | | | | |
| Number | 47 | 9 | 1 | 57 |
| Frequency | 82, 4 | 15, 7 | 1, 7 | 100 |

Only 35% of SS patients have a negative CRP (<6 mg/l) against 82% in AA controls. On the other hand, 35% of SS patients have a CRP ≥96 mg/l against only 1.7% of cases in AA controls, which indicates an increase in acute inflammation in sickle cell disease.

## DISCUSSION

The sickle cell population of this study was young children with an average age of 13 years, which is in agreement with the results of several authors in Mali and Morocco.[13-15] In addition, the percentage of sickle cell patients decreased with age, this could be explained by the early mortality of the subjects. Indeed, mortality before the age of 5 reaches 50% in the absence of appropriate health care.[16]

The sex ratio (M/F) was 1.73, which was consistent with studies carried out in Dakar and in Niger, which respectively found sex ratios (M/F) of 1.02 and 1.2.[17,18] By cons, in Brazzaville, in the Democratic Republic of Congo and in Senegal were observed a female predominance with respectively a sex ratio (M/F) of 0.8, 0.9 and 0.75.[15,19,20] These differences would be related to the socio-demographic data of each country, because the transmission of sickle cell disease is not linked to sex.[21]

His hemoglobin level fell very significantly in SS subjects, which may explain the sickle cell anemia.[14,15,20]

The results of the study showed a very significant increase in the number of leukocytes in SS subjects. This increase affects all types of leukocytes, namely neutrophils, eosinophils, lymphocytes and monocytes. These observations were consistent with some data from the literature. The noted hyperleukocytosis could be the result of bone marrow hyperproduction associated with permanent demargination from the marginal pool to the circulating pool.[22] This uncorrected leukocytosis is often very important in homozygotes.

The elevation of CRP argued in favor of an inflammatory reaction likely to increase the number of leukocytes, which was in agreement with the results of Doupa which showed a positive correlation between the two parameters.[15] However, this result differed from the case of malaria in which the increase in CRP is consistent with that of the cells of acute inflammation, which were neutrophils, and decreases with those of chronic inflammation, namely lymphocytes and monocytes.[23] In sickle cell disease, it looks like there was both acute and chronic inflammation.

The mean number of blood platelets was very significantly low in SS subjects. Similar results were observed in the littérature.[13,15] Regarding renal function, serum uremia and serum creatinine were significantly higher in SS subjects, which is indicative of impaired renal function. However, all observed values remain within physiological limits. This was also observed in Niger and Cameroon.[24,25]

Serum uric acid was significantly lower in SS subjects. Indeed, in sickle cell disease, there was a lysis of red blood cells (anucleate cells). However, this loss of red blood cells was actively compensated by an increased production of young red blood cells by the bone marrow to compensate for the anemia.[26] This hyperactivity of the bone marrow

could explain a better use of nucleic acids for the multiplication and differentiation of erythroblasts, which would reflect a decrease in their degradation, and therefore a decrease in the level of uric acid. However, some authors have observed an increase in uricemia in SS.[24]

Hepatic markers did not show liver damage in SS since ALT transaminase, a specific liver marker, did not increase. Only AST increased, indicative of general cytolysis. All observed values are within physiological limits.

***Limitations***

The sampling of patients who come to the hospital after taking medication or herbal treatments.

## CONCLUSION

In sickle cell disease, there were inflammation, cytolysis and kidney damage accompanied by anemia and thrombocytopenia. These hematological and biochemical parameters could serve as predictive biomarkers of complications and evaluations of the effectiveness of various proposed remedies in the treatment or prevention of sickle cell crises. Also, these markers could be used to test the effectiveness of medicinal plants used to treat sickle cell crises.

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