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Electrolytic and Oxidative Stress Profile of Sickle Cell Anaemia Patients in Cameroon: The Effect of Some Extrinsic Factors

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Authors' contributions

This work was carried out in collaboration between all authors. Author KFCB conducted the study and assays, managed the literature searches and wrote the first draft of the manuscript. Author BNPC designed the research, co-directed the research work as well as the statistical analysis study. Author CB facilitated the contact between principal investigator and SCA patients and helped explaining the importance of such research to patients. Author NLF assisted in conducting the assays. Author PCA co-directed the research work and author GD supervised the research. All authors read and approved the final manuscript.

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ABSTRACT

Sickle cell anaemia is a hereditary blood disease characterized by the presence of haemoglobin S in red blood cells. It affects over 2 million people in Cameroon. Haemoglobin S can induce the oxidative stress and changes in electrolyte level in patients. The aim of this study was to measure

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the serum electrolytes Ca, Mg, K, P, Fe, Zn, Cu, Se and certain stress markers (Malondialdehyde , FRAP and catalase) in sickle cell patients.
Forty SS patients and forty AA individuals (control) were selected and given questionnaires to gather information on their demographic characteristics, health states and eating habits. The Ca, Mg, K, P, Fe, Zn, Cu and Se elements as well as certain stress markers were assessed in their serum using the atomic absorption spectrophotometry and colorimetric methods respectively.
The results obtained showed that SS patients present a deficiency in micronutrients and a high state of stress. The regular consumption of foods such as fish, milk and rice by sickle cell patients significantly increased the normal rate of electrolytes. On the contrary, hyperthermia, intense sport and alcohol consumption lowered their normal rate.
Conclusively, the assessment of electrolyte levels and oxidative stress should be taken into consideration in the management of sickle cell disease.

Keywords: Sickle cell disease; electrolytic profile; Malondialdehyde; catalase; FRAP; food.

ABBREVIATIONS

FRAP : Ferric Reduction Antioxidant Power
Hb : Haemoglobin
MDA : Malondialdehyde
ROS : Reactive Oxygen Species
SCA : Sickle Cell Anaemia
SCP : Sickle Cell Patient

1. INTRODUCTION

Sickle cell anaemia (SCA) is a hereditary disease characterized by altered haemoglobin (Hb) [1]. It is caused by a mutation at the 6^{th} codon of the beta chain of chromosome 11 and translated on the protein level by the substitution of glutamic acid by valine [2], thus transforming the Hb A into Hb S. It is the most widespread genetic disease in the world affecting more than 50 million people [3], including 38 million in sub-Saharan Africa [4]. In Cameroon the statistics are alarming. SCA causes about 4,000 deaths each year. Currently, the country alone has two of the fifty million SCA in the world. Of the 200,000 sickle-cell patients (SCP) surveyed, half die before the age of 5 [4], making it a real public health problem. The prevalence of the disease in Cameroon is between 2-3% [5]. The management of the disease is based on the treatment of vaso-occlusive attacks, prevention of seizure factors, folate supplementation, preventive treatment of pneumococcal and meningococcal infections, blood transfusion in severe anaemia or severe infection and the transfusion-bleeding to reduce the proportion of haemoglobin S [6].

The haemoglobin S of SCP induces changes in the membrane permeability of erythrocytes resulting in a change in electrolyte level in the latter. Indeed, deoxygenated erythrocytes in the spleen increase sodium and potassium fluxes, with cellular gain of Na^+ and loss of K^+ [7]. The homeostasis of red blood cells is controlled partly by the dynamic equilibrium between ions, especially free calcium (Ca^{2+}) and magnesium (Mg^{2+}). Calcium stimulates the flow of potassium (K^+) and loss of cellular water via the Ca^{2+}-K^+ channel (Gardos channel), thus promoting dehydration, while magnesium inhibits the K^+-$C1^-$ co-transport system, decreases the loss of potassium and consequently promotes hydration of the cell. The low level of total magnesium in the red blood cells of sickle-celled anaemia patients is therefore linked to an increase in potassium efflux, leading to cell dehydration [8]. SCP may also have microelements deficiencies [9], probably due to increased nutrient requirements, decreased dietary intake, intestinal malabsorption and high catabolism [10]. Complications such as sickling, vaso-occlusive seizures and ischemia observed in sickle cell patients are cyclic in nature and induce the generation of reactive oxygen species (ROS) leading to oxidative stress [11]. Although the level of oxidative stress is high in SCA, marker levels may also be related to other extrinsic factors such as diet, physical activity, alcohol, heat and the environment [12,13]. These extrinsic factors also contribute to the onset of sickle cell seizures [14,15] and may therefore have an impact on electrolyte level.

Several studies have been carried out on electrolytes in SCA, mainly in Nigeria, India, Brazil and the USA, but only took into account two and five elements simultaneously. In Cameroon, data on the electrolyte profile and sickle cell stress are quite rare. Moreover, although the disease is classified as a public health problem in Cameroon, SCP are still marginalized, with very few access to care that remains costly. However, data on the electrolyte

profile and the level of oxidative stress in these patients could contribute to a better management of the disease in terms of quality and cost, hence the motivation to carry out this study.

Thus, the general objective of this study is to contribute to the better management of SCP by studying their electrolyte and antioxidant profile. More specifically,

- Evaluate the influence of sickle cell status on the electrolyte profile and on the state of oxidative stress of sickle cell patients,
- Evaluate the influence of extrinsic factors (alcohol, tobacco, diet, water intake, fever) and intrinsic factors (sex and age) on the electrolyte profile and oxidative stress of sickle cell patients in the city of Yaoundé.

2. MATERIALS AND METHODS

2.1 Ethical Consideration, Type of Study and Patient Selection

This study was approved by the Cameroon Bioethics Initiative (CAMBIN) and the ethical clearance was obtained under the reference number CBI/387/ERCC/CAMBIN. Patients gave "informed consent". We carried out a cross-sectional study using, on one hand, a questionnaire administered to all participants and on the other hand the evaluation of electrolytes and some markers of oxidative stress in serum.

Sampling was probabilistic with individuals of both sexes selected at random. 80 participants were selected at random (40 SS and 40 AA). Were included in this study sickle cell homozygotes (SS) confirmed and AA individuals, all one year old at least, with voluntary participation in the study and with the informed consent signed by the participant or parent obtained. The heterozygotes (AS), those with renal insufficiency, chronic diarrhea, have received blood transfusion less than 72 h ago, less than one year old, those receiving electrolytes as food supplements and pregnant women were not included in this study.

2.2 Data and Blood Collections

Each participant received a questionnaire that was completed under our control. The questionnaire consisted of three main parts: sociodemographic parameters (gender, age, occupation, ethnicity); the anthropometric parameters and the state of the participant (height, weight, temperature, last meal of the day, date of the last crisis, date of the last blood transfusion, medication in progress, other health problems); participants' dietary habits (food, alcohol, tobacco, food supplements and water). The temperature, the weight and height were also taken. Blood sample collection was done with the help of the hospital personnel. Five millilitres (5 mL) of blood each were taken and introduced into previously labelled dry tubes, followed by centrifugation at 3500 rpm for 10 minutes and the obtained serum was introduced into eppendorf tubes and stored at -20 °C until analysis. The haemolyzed samples were discarded.

2.3 Parameters Measurement

2.3.1 Biochemical parameters

Calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), Iron (Fe), copper (Cu), zinc (Zn) and selenium (Se) concentrations were measured using atomic absorption spectrophotometry [16]. Briefly, the blood sample firstly underwent extraction using "aqua regia" (75% concentrated nitric acid and 25% concentrated hydrochloric acid). One millilitre (1 mL) of serum was mixed with 10 mL of regal water and then introduced into a beaker. The mixture was heated until complete evaporation of the nitrous vapour. It was then allowed to cool the mixtures introduced into a 50 mL graduated flask and the flask completed to the mark with distilled water and homogenized. After reading the optical densities, the calibration curve was plotted automatically by the computer connected to the spectrophotometer, using a standard range for each element. The calibration curve helped us to calculate the concentrations of the elements in the different samples according to Beer-Lamber's law: $DO = \varepsilon lc$.

2.3.2 Total antioxidant activity by ferric reducing antioxidant power assay (FRAP)

The FRAP was determined using the method described by Benzie and Strain [17]. This test measures the reduction of ferric ion to the ferrous form in the presence of antioxidant compounds. The fresh FRAP reagent consisted of 500 mL of acetate buffer (300 mM and pH 3, 6), 50 mL of 2, 4, 6- Tri (2-pyridyl)-s-triazin (TPTZ) (10 mM) and 50 mL of $FeCl_3 \cdot 6H_2O$ (50 mM). The colorimetric measurement was performed at 593 nm and the

reaction was monitored up to 12 minutes on 75 µL of each extract and 2 mL of FRAP reagent. The ascorbic acid was used to draw a standard curve and the Butylated Hydroxyl toluene (BHT) was used for the comparison. The absorbance was read at 593 nm.

2.3.3 Catalase measurement

The activity of catalase was determined according to the method of Sinha [18]. One hundred microlitres (100 µL) of the various test solutions or control freshly prepared were introduced into test tubes containing 900 µL of phosphate buffer (0.01M, pH 7). After homogenization, 400 µL of hydrogen peroxide (200 mM) were added in order to induce the reaction and after 60 s, 2000 µL of a dichromate-acetic acid solution were added to terminate the reaction. The mixture was heated to 100°C for 10 minutes and the absorbance measured at 530 nm. The activity of catalase was determined using the following formula:

$$CA = \frac{OD}{1{,}5118 \times Vs \, X \, C_{proteins}}$$

CA = catalase activity (UI/mg of proteins)

OD = optical density/min

[proteins] = Concentration of proteins in the different serum (mg/ml)

Vs = Volume of test solution/control

2.3.4 Malondialdehyde (MDA) measurement

The method used for the MDA assay was that of Folch et al. [19]. One hundred microlitres (100 µL) of distilled water and 100 µL of test solution or freshly prepared test solution and 2000 µL of MDA solution (TCA-TBA-HCl) were introduced into different test tubes. The mixture was then homogenized and heated to 100°C for 15 minutes. After cooling, centrifugation was carried out at 3000 rpm for 5 minutes. The supernatant was collected in order to read the optical density at 532 nm. The MDA value of the tissue in mM was then calculated using the molar extinction coefficient of 1.56 × 105 /M.cm.

2.4 Statistical Analysis

The data were processed in the Excel software and analyzed with SPSS (Statistical Package for Social Sciences) version 16.0. The results were expressed as mean ± SD, the chi-square test was used to compare the means and the P < .05 value was considered significant.

3. RESULTS

Table 1 shows the mean serum concentrations of the macroelements and microelements in SS group compared to AA group. There was a significant (P < .05) decrease in zinc, iron and selenium microelements and magnesium, calcium and phosphorus macroelements in SCP (SS) compared to the healthy population (AA), but rather a significant increase (P < .05) of copper was observed in SS patients compared to healthy AA. Overall, this table shows a deficiency in microelements in sickle cell patients compared to control.

The serum concentrations of the oxidative stress markers in the two groups are presented in Table 2. Sickle cell patients had a higher state of oxidative stress than controls. There was a significant decrease in catalase activity (P = .01) and an increase although not significant (P = .504) for MDA in sickle cell patients compared to AA control individuals.

Table 1. Impact of sickle cell disease on macroelements and microelements

Groups	Macroelements (*mM*)			
	K	**Ca**	**Mg**	**P**
SS	5.47 ± 1.72	1.21 ± 0.89*	1.12 ± 0.20*	2.76 ± 0.73*
AA	5.02 ± 1.31	2.18 ± 1.3	1.32 ± 0.14	3.94 ± 1.66
P value	.194	.001	.000	.000
	Microelements (*mM*)			
	Cu	**Zn**	**Fe**	**Se**
SS	29.71 ± 11.81*	0.98 ± 0.46*	74.39 ± 43.45*	0.47 ± 0.42*
AA	19.21 ± 11.33	27.67 ± 9.81	99.92 ± 45.27	1.92 ± 0.84
P value	.001	.000	.012	.000

*Independent t- test at P < .05. SS=Sickle cell patients. AA= health people. *=significant difference at 0.05*

Table 2. Impact of sickle cell disease on the oxidative stress

Groups	FRAP (*µM*)	MDA (*µg/mL*)	Catalase (*Ul/mg of protein*)
SS	142.06 ± 34.71*	1.18 ± 1.12	3.44 ± 1.06*
AA	118.50 ± 40.08	1.01 ± 0.82	8.42 ± 3.54
P value	.016	.504	.000

*Independent t- test at P<.05. SS=Sickle cell anaemia patients. AA= health people. *=significant difference at.05 in column. FRAP = Ferric Reduction Antioxidant Power; MDA = Malondialdehyde*

The percentages of the normal rate levels of the biochemical parameters assayed according to the practice or not of a sporting activity by sickle cell anaemia are presented in Table 3. It is evident that the practice of intense sports by sickle cell patients significantly decreased (P <.05) the percentage of normal electrolyte and MDA levels compared to those who do not practice sport.

Table 4 shows effects of some extrinsic factors on the normal rate percentage of some electrolytes and oxidative stress markers in sickle cell anaemia patients. As concerns water consumption, at least 1.5 L of water consumed per day by sickle cell patients significantly increased (P < .05) the percentage of normal electrolyte and MDA levels compared to those who drank less per day.

The percentages of normal levels of biochemical parameters measured as a function of the patient's temperature at the time of blood sampling are presented in Table 4. It appears from this table that the rise in temperature significantly dropped (P < .05) the percentage of the normal electrolyte and MDA levels. In general, sickle cell patients with normal temperature (36.5 ≤ t ≤ 38°C) had significantly higher electrolyte percentage (P < .05) than patients with fever (t > 38°C) C) at the time of collection.

Table 4 shows the percentages of normal levels of biochemical parameters measured in SS patients according to their health state (in crisis or not) at the time of sampling. It was found that sickle-cell anaemia significantly decreased (P < .05) the percentages of normal electrolytes levels in patients.

Alcohol effects were equally studied and are presented in Table 4. It appears that alcohol consumption by SS patients significantly decreased (P < .05) the percentage of normal electrolyte level.

The percentages of normal levels of biochemical parameters as a function of the frequency of fish consumption show that the regular consumption of fish by sickle cell patients significantly increased (P < .05) the percentages of the normal levels of Mg, P, Cu, Fe, Se and MDA elements (Table 4).

The relationship between normal electrolyte level and bread, beans and rice consumption was also evaluated. It appears on Table 4 that the consumption of rice and bread regularly by sickle cell patients significantly increased (P < .05) the percentages of the normal rates of electrolytes and MDA whereas the consumption of beans decreases these parameters.

Table 3. Influence of some non consuming extrinsic factors on the normal rate percentage of some electrolytes and oxidative stress markers in sickle cell anaemia patient

Normal rates of parameters according to the practice or not of sport in SS patients								
Groups	**K**	**Ca**	**Mg**	**P**	**Cu**	**Fe**	**Se**	**MDA**
Sport (%)	33	14	35	17	7	33	23	20
No sport (%)	67	86	65	83	93	67	77	80
P value	.001	.001	.00	.001	.00	.00	.00	.00
Normal parameters rates according to the state of crisis in SS patients								
In crisis (%)	17	43	23	17	21	0	15	20
Non in crisis (%)	93	57	77	83	79	100	85	80
P value	.001	.008	.00	.014	.00		.00	.00

Chi-square test. Significance threshold P <.05. Reference value of some parameters: Copper: 70-150 µg/dL. Ionized calcium: 1.03-1.23 mmol/L. Magnesium: 1.5-2 mEq/L. Potassium: 3.5-5 mmol/L. Sodium: 135-145 mmol/L. Zinc: 70-100 µmol/L. Selenium: 0.01-0.04 ppm. Iron: 0.011 et 0.034 mmoles/l. MDA: 0.079 nmol/ml

Table 4. Influence of some consuming extrinsic factors on the normal rate percentage of some electrolytes and oxidative stress markers in sickle cell anaemia patients

Groups	K	Ca	Mg	P	Cu	Fe	Se	MDA
	Normal rates of parameters according to water consumption in SS patients							
0.5-1.5 L/day (%)	8	28	29	16	28	0	23	7
1.5-3 L/day (%)	50	29	47	50	50	33	38	53
3.-4.5 L/day (%)	42	43	24	34	22	67	39	40
P value	.000	.007	.00	.017	.00	.05	.00	.00
	Normal parameters rates according to body temperature in SS patient							
36.5-38°C (%)	92	86	88	100	93	100	85	93
>38°C (%)	8	14	12	0	7	0	15	7
P value	.015	.008	.00		.00		.00	.00
	Normal parameters rates according to alcohol consumption in SS patients							
Alcohol (%)	16	14	23	50	29	33	23	33
No alcohol (%)	84	86	77	50	71	67	77	67
P value	.001	.008	.00	.9	.00	.00	.00	.00
	Normal parameters rates according to fish consumption in SS patients							
fish regularly (%)	58	57	77	83	71	67	54	67
Fish rarely (%)	42	43	23	17	29	33	38	33
P value	.001	.007	.00	.014	.00	.083	.00	.00
	Normal parameters rates according to fish consumption in SS patients							
Rice all days (%)	17	14	0	17	7	0	15	20
Rice regularly (%)	58	29	71	50	72	33	62	53
Rice rare (%)	17	57	29	33	21	67	23	20
No rice (%)	8	0	0	0	0	0	0	7
P value	.000	.014	.00	.025	.00	.083	.001	.00
	Normal parameters rates according to bread consumption in SS patients							
Bread all days (%)	83	72	94	50	79	100	84	86
Bread regularly (%)	17	14	6	17	14	0	8	7
No bread (%)	0	14	0	33	7	0	8	7
P value	.001	.007	.00	.000	.00		.000	0.00
	Normal parameters rates according to beans consumption in SS patients							
Beans all days (%)	0	0	24	16	0	0	8	0
Beans regularly (%)	17	14	41	17	21	0	15	7
Beans rarely (%)	25	29	35	17	50	0	46	52
No beans (%)	58	57	0	50	29	100	31	40
P value	.001	.007	.00	.035	.00		.000	.00

1-2 times / week = rarely; 3-5 times / week = regularly. Chi-square test. Significance threshold P < .05. Reference value of some parameters: Copper: 70-150 µg/dL. Ionized calcium: 1.03-1.23 mmol/L. Magnesium: 1.5-2 mEq/L. Potassium: 3.5-5 mmol/L. Sodium: 135-145 mmol/L. Zinc: 70-100 µmol/L. Selenium: 0.01-0.04 ppm. Fer: 0.011 et 0.034 mmoles/l. MDA: 0.079 nmol/ml

It appears on Tables 5 and 6 that no significant differences exist between men and women for all biochemical parameters measured in SS patients following gender and age (intrinsic factor).

Table 5. Biochemical parameters variation as a function of gender in SS patients

Elements	Women (N=20)	Men (N=20)	P value
K (mM)	5.47 ± 1.65	4.55 ± 1.70	0.093
Ca (mM)	0.75 ± 0.79	0.75 ± 0.91	1.000
Mg (mM)	0.72 ± 0.46	0.68 ± 0.48	0.807
P (mM)	2.22 ± 0.65	2.30 ± 0.98	0.777
Cu (µM)	28.71 ± 13.91	29.72 ± 9.99	0.816
Zn (µM)	0.41 ± 0.51	0.68 ± 0.75	0.216
Fe (µM)	74.50 ± 44.34	73.30 ± 43.77	0.932
Se (µM)	0.13 ± 0.35	0.12 ± 0.33	0.898
MDA (µg/mL)	0.84 ± 1.30	0.58 ± 0.77	0.453
FRAP (µM)	134.05 ± 37.65	149.95 ± 30.64	0.162
Catalase (UI/mg of proteins)	2.79 ± 0.98	3.32 ± 1.11	0.129

Independent T-test. Significance threshold P < .05.

Reference value of some parameters: Copper: 70-150 µg/dL. Ionized calcium: 1.03-1.23 mmol/L. Magnesium: 1.5-2 mEq/L. Potassium: 3.5-5 mmol/L. Sodium: 135-145 mmol/L. Zinc: 70-100 µmol/L. Selenium: 0.01-0.04 ppm. Fer: 0.011 et 0.034 mmoles/l. MDA: 0.079 nmol/ml

Table 6. Biochemical parameters variation as a function of age in SS patients

Elements	Age range (years)				
	1-10 (N=4)	10-20 (N=15)	20-30 (N=17)	30-40 (N=3)	P value
K (mM)	4.75 ± 2.63	5.14 ± 1.88	5.12 ± 1.36	4.67 ± 2.31	0.803
Ca (mM)	0.50 ± 0.58	0.67 ± 0.82	0.71 ± 0.77	2.00 ± 1.00	0.083
Mg (mM)	0.75 ± 0.50	0.60 ± 0.51	0.73 ± 0.46	1.00 ± 0.00	0.743
P (mM)	2.50 ± 0.58	2.40 ± 0.99	2.20 ± 0.77	1.67 ± 0.58	0.670
Cu (µM)	27.00 ± 12.36	29.33 ± 13.66	31.53 ± 10.64	19.00 ± 11.53	0.566
Zn (µM)	.	0.60 ± 0.51	0.46 ± 0.52	1.33 ± 1.53	0.082
Fe (µM)	65.25 ± 49.90	63.73 ± 36.61	85.65 ± 41.12	52.33 ± 72.54	0.267
Se (µM)	0.33 ± 0.58	0.10 ± 0.32	0.13 ± 0.35	.	0.800
MDA (µg/mL)	0.25 ± 0.50	0.73 ± 1.22	0.88 ± 1.09	.	0.737
FRAP (µM)	121.5 ± 33.9	143.6 ± 27.1	140.3 ± 39.5	165.5±57.3	0.487
Catalase (UI/mg dof proteins)	3.50 ± 1.29	3.07 ± 1.22	2.94 ± 0.77	4.00 ± 0.00	0.182

Independent T-test. Significance threshold P < .05.

Reference value of some parameters: Copper: 70-150 µg/dL. Ionized calcium: 1.03-1.23 mmol/L. Magnesium: 1.5-2 mEq/L. Potassium: 3.5-5 mmol/L. Sodium: 135-145 mmol/L. Zinc: 70-100 µmol/L. Selenium: 0.01-0.04 ppm. Fer: 0.011 et 0.034 mmoles/l. MDA: 0.079 nmol/ml

4. DISCUSSION

In this study, SCA significantly affected the levels of electrolytes and some stress markers in SS patients, compared to the healthy AA control population. The results show a significant decrease in the macroelements magnesium (Mg), phosphorus (P) and calcium (Ca) and the microelements zinc (Zn), iron (Fe) and Selenium (Se), against a significant increase in copper (Cu) and potassium (K) (although not significant for the latter) in sickle cell patients compared to the AA control population. That is, SS patients of Yaoundé suffer from microelements deficiency. The lowest magnesium level observed in SCP could be a consequence of renal magnesium losses [20] or linked to a diet low in magnesium [8]. Indeed, it has been observed in this study that SCP rarely consumes chocolate, vegetables and beans, which are foods rich in magnesium [21]. These results are in agreement with other works [9,22] on the variations of serum electrolytes in SCA compared to healthy controls. The low level of phosphorus in SCP could be due to low renal reabsorption of phosphate, a result of increased secretion of parathyroid hormone in them [23]. Hypocalcemia observed in sickle cell patients could in turn be due to a decrease in

Ca^{2+}-Mg^{2+}ATPase, reduced intestinal absorption of calcium and reduced synthesis of vitamin D [24]. Indeed, in SCA, there is generally a decrease in the activities of Ca-ATPase, Na-ATPase and K-ATPase [25]; the decrease in Ca^{2+} -Mg^{2+}ATPase activity causes more calcium to enter the erythrocytes resulting in a low plasma concentration. These results corroborate those of a recent work [26] that evaluated the serum levels of calcium, sodium, potassium and phosphorus in sickle cell SS patients compared with AA controls, demonstrating significant decrease in calcium in SCP. The non-significant increase in potassium in SS compared to AA is identical to the other results [27], which revealed a non-significant increase in serum potassium in SCP compared to controls. This increase in potassium may be due to activation of the Gardos channel by the excessive intake of calcium into the erythrocytes, leading to the release of K^+ [8].

The general deficiency in microelements observed in SCP may be due to inadequate absorption of these elements because of chronic pain, reduced appetite and chronic haemolysis. Reduced zinc levels may be due to increased requirements in SS, an increase in urinary excretion of zinc itself caused by hypoxanthinuria [28]. The low selenium content observed in SS patients may be related to increased oxidative stress [9]. The lower iron content in the SS than in the control population may be explained by a low nutritional status. Indeed, we observed that SCP rarely consume meat and vegetables, and most do not consume fruits that are foods rich in iron [21]. This result may also be related to the presence of intestinal parasites, malabsorption syndrome and increased urinary excretion of iron, which are very present in SCA [29]. About one-third of hemolysis in SCA is intravascular [30]; the resulting excessive urinary iron loss could lead to a decrease in iron in the body.

The increase in copper observed in SS may be due to the observed decrease in zinc. Indeed, copper and zinc compete for similar binding sites on certain proteins such as albumin and metallothionine [31], therefore a decrease in zinc can lead to an increase in copper [27]. These results corroborate previous works [9,32,27] which presented significantly lower serum levels of Se, Zn and Mg against high copper levels in SCA patients compared with healthy control individuals. The decrease of catalase activity and the increase of the lipid peroxidation all significant, observed in SCA are the sign of a state of oxidative stress in the latter. The increase in lipid peroxidation in SS patients may be due to the auto-oxidation of HbS coupled with the decompartmentalization of iron observed in the latter [33]. Indeed, the increased auto-oxidation of HbS, which produces superoxide radicals, also results in the formation of a pathological amount of hydrogen peroxide (H_2O_2) which, in the presence of free iron, produces free radicals which in turn cause serious damage to membrane proteins and lipids, leading to oxidation of thiols and lipid peroxidation [33,34]. The free iron produced in SCA due to decreased haeme synthesis can also act as a Fenton reagent with hydrogen peroxide and give rise to the superoxide, peroxide and hydroxyl radicals which consequently leads to the initiation of lipid peroxidation, hence the increased the production of MDA in SCA patients [35,36]. This excessive production of ROS is also the cause of decreased catalase activity in SCA patients, as ROS, particularly H_2O_2, are able to inhibit the action of antioxidant enzymes including catalase [35,36]. H_2O_2 increases the phosphorylation of protein kinase B (Akt kinase), thus causing the phosphorylation of the transcription factor FoxO1, the increase of which inhibits the expression of catalase [37]. These findings corroborate those of recent studies [36,38,39, 40], which have demonstrated a significant decrease in catalase activity and an increase in MDA production in SCP compared to healthy controls.

The results from this study show that there is no significant difference in the average levels of electrolytes and markers of oxidative stress according to gender and age. This implies that electrolyte levels, as well as markers of oxidative stress in SCP do not depend on gender or age. This could be explained by the fact that SCA is a genetic disorder with autosomal recessive transmission, implying that it affects both girls and boys alike. If half of the SCPs die before the age of five, the other half, well attended, can live up to old age. These results corroborate those obtained by Idonije et al. [9] who established that serum levels of microelements in sickle cell patients were independent of gender and age.

For extrinsic factors, the results show that non-sporting SS patients had significantly higher levels of electrolytes and MDA compared to those who practiced intense sport. This could be explained by the fact that shortness of breath causes desaturation of Hb in O_2, leading to the acceleration of sickling and consequently to the

production of ROS and to ion perturbation [41]. Similarly, the intake of at least 1.5 L of water per day significantly increases the percentage of normal electrolytes levels; which could be explained by the fact that hydration increases the fluidity of the blood and limits the sickling of the red blood cells. The results also show that elevation of temperature significantly decreases the percentage of normal electrolyte and MDA levels; this is due to the fact that fever causes dehydration and the formation of inflammatory proteins that slow down blood flow [41].

The regular consumption of fish, milk, rice and bread, as well as the rare consumption of beans by the patients, significantly increases the percentages of the normal electrolytes and MDA levels. This could be explained by the fact that dairy products, fish and cereals are rich in macro and microelements [21]. Moreover, the percentages of the normal electrolytes and MDA levels significantly reduce in patients consuming alcohol and could be explained by the fact that alcohol is a dehydrating agent, which can consequently lead to seizures [41].

5. CONCLUSION

These results suggest that patients with sickle cell anaemia in the city of Yaoundé suffer from microelement deficiency and are under high stress, not related to intrinsic factors, but may be linked to certain extrinsic factors such as alcohol, temperature, the state of crisis, water and some dietary consumption. Thus, sickle cell patients should avoid alcohol consumption, intense sport and fever. They should drink at least 1.5 L of water per day and have a good dietary. A full consideration of all the above factors could enhance SCA patients care.

DATA AVAILABILITY

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

CONSENT

Patients gave “informed consent”.

ETHICAL APPROVAL

The study protocol was approved by the Cameroon Bioethics Initiative and the ethical clearance was obtained under the reference number CBI/387/ERCC/CAMBIN.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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