

**Protein-energy status and oxidative stress of a group of patients with sickle cell disease in
Yaoundé - Cameroon: Effect of some intrinsic and extrinsic factors**

ABSTRACT

Background: SCA is a systemic disease which affects more than 50 million people in the world. The gene mutation results in the cause of the synthesis of abnormal haemoglobin S (HbS) which is a pro-oxidant machine and induces ROS production. In addition, sickle cell patients are deficient in body composition parameters. The aim of this study is to contribute to a better management of sickle cell anaemia patients by evaluating the relationship between protein-energy status and the state of oxidative stress.

Methods: Hundred participants were recruited and divided into two groups (52 sickle cell patients and 48 healthy participants). The investigation on their Body Mass indices and their nutritional status were conducted through a questionnaire. Several biochemical parameters related to proteo-energy deficiency and oxidative stress were assessed such as albumin and transferrin, Malondialdehyde (MDA), total antioxidant capacity (FRAP), reduced glutathione (GSH), glutathione peroxidase (GPX), catalase (CAT) and Superoxide dismutase (SOD) activities using standard methods.

Results: BMI and MDA was significantly less in SS group compared to the healthy population. Opposite observations were done to other markers investigated including GSH, CAT, SOD and GPX activities and FRAP. The Albumin level increased significantly while transferrin values remained comparable. The oxidative stress and protein-energy status parameters presented

negative correlations. Factors such as age, hydration, consumption of meat and fruit affected the biochemical parameters investigated.

Conclusion: These results suggest that sickle cell patients in Yaoundé-Cameroon are underweight with higher oxidative stress status. Moreover, they are under stress; nevertheless, they have a good protein-energy status.

Key words: sickle cell disease; oxidative stress; protein-energy malnutrition.

BACKGROUND

Haemoglobinopathies are genetic diseases in which there is an inheritance of abnormal haemoglobin. Sickle cell anaemia (SCA) represents the first genetic disease worldwide [1]. It is caused by the mutation of one of the chains of haemoglobin where there is a substitution of glutamic acid by valine in position 6 of the beta chain of haemoglobin which is coded by chromosome 11 [2]. SCA is especially common in people of the Caribbean, African and Mediterranean origin [3]. This disease is spreading in the world as a result of the increase in migration and the geographical spread of some disease genes. More than 500,000 of SCA children are born worldwide, with 300,000 in Africa and half of these children die in Africa before the age of 5 years [1]. In Cameroon, SCA killed about 4,000 people every year; and currently, the country has about two million among the 50 million sickle cell patients identified worldwide [4] with a prevalence 2-3% [5].

The physiopathology of SCA includes polymerization of deoxygenated HbS. This molecule polymerizes and alters the normal structure of red blood cells, thus weakening these cells and causing intracellular lyses responsible for the release of haemoglobin in the plasma [6]. Sickled haemoglobin (HbS) is also called a pro-oxidant machine. These independent events can interact with the cell membrane, creating reactive oxygen species (ROS), altering antioxidants profile [7],

which lead to an oxidative stress. In addition, studies of supplementation with protein and calories have shown clear clinical improvement and growth in children with sickle cell anaemia [8], thus indicating the existence of a protein-energy malnutrition deficiency. A study conducted in Brazzaville (Congo) has proven that children with sickle cell anaemia are characterized by a growth delay, a decrease in body weight, fat, muscle mass and body mass index in all the age groups [9]. Besides age and sex, other extrinsic factors such as sport, for example, could also contribute to worsen the health of patients [10]. Furthermore, previous study conducted on the electrolytic profile status on patients suffering from SCA [11] in Yaoundé-Cameroon demonstrated that these patients have asthenia, low body weight and also anxiety. This study aims to evaluate the relationship between protein-energy status and oxidative stress status of patients with sickle cells disease in order to contribute to a better management of these patients

MATERIAL AND METHODS

Ethical Considerations

This study was approved by the Regional Ethics Committee of research on human health of the Centre (CRERSH-CE); that issued an ethical clearance under the reference No. 0283/CRERSHC/2016 on behalf of the Ministry of public health of Cameroon under the reference No. 0287/AP/MOH/SG/DRSPC. Then, before conducting the study, the general director of the central hospital of Yaoundé authorized the recruitment of patients in his hospital under the reference No. 066/MOH/SG/DHCY/CM. Before recruiting, patients were informed of the objectives of the study and were requested the written informed consent according to the declaration of Helsinki.

Type, duration and sites of study

A joined cross-type case-control study was carried out. First of all, a questions form helped us to have certain information permitting us to calculate anthropometric parameters (BMI) and to have the feeding habits of the persons taking part. The evaluation of energy-protein profile and the state of oxidative stress was done subsequently through the determination of the specific biochemical parameters. This study lasted for six (06) months and took place during the period of August 15, 2016, to January 15, 2017.

This study was carried out on two different sites, the central hospital of Yaoundé, the Haematology service for the collection of blood samples from patients and the laboratory of Biochemistry, Department of Biochemistry and Physiological Sciences of the Faculty of Medicine and Biomedical Sciences (FMSB), University of Yaoundé I, for the different determination of the biochemical parameters.

Recruitment of patients

Sampling was probabilistic, and individuals of both sexes were recruited randomly. The size of the sample (100 individuals) was calculated from the formula of Lorenz. Were included in this study, patients confirmed to be homozygous SCA and confirmed to be healthy homozygous individuals (AA), all ages of at least one year. Were not included in this study, heterozygous individuals (AS), homozygous individuals AA or SS with kidney insufficiency, inflammatory syndrome or those who have received blood transfusion in less than 72 h before dialyging.

Blood sampling and data collection

A questionnaire form was submitted to patients. It consisted of 4 different parts: socio-demographic data (age, sex, region of origin, place of residence, temperature, weight and size); general information about the energy-protein malnutrition, the state of oxidative stress, the

management of the patient (date of the last crises, date of the last blood transfusion, medicines, other Pathology) and finally their eating habits. The temperature and weight of patients were measured using respectively a thermometer and a scale, a measuring rod helped in measuring the size. Blood collection was ensued with the assistance of the medical personnel using dry tubes. After centrifugation at 3500 rpm for 15 min, the serum obtained was stored in “creotubes” at - 20° C until the determination of the various biochemical parameters.

Determination of the biochemical parameters

The energy-protein profile included the determination of several biochemical parameters including albumin and transferrin according to standard method (Cypress Kits, Belgium). The oxidative status markers were evaluated through the determination of lipid peroxidation [12], reduced glutathione [13], total antioxidant capacity [14], catalase [15] superoxide dismutase [16] and glutathione peroxidase.

STATISTICAL ANALYSES OF DATA

The normality of our main variables was examined in order to estimate the type of distribution [17]. At the end of this estimate, parametric tests have been used. The results were expressed as a mean $\pm$ standard deviation, the Chi-square test was used to compare the frequencies, and the Independent t-test was used to compare means. The Pearson correlation helped in estimating the level of association between quantitative variables. Data were processed in the Excel and analyzed with the SPSS software (Statistical Package for Social Sciences) version 16.0. The value $P < .05$ was considered to be significant.

RESULTS

Out of the 100 participants, 27 men (52 %) and 25 women (48 %) represented the SCA patients while 17 men (35 %) and 31 women (65 %) represented the healthy control. 44 % were

adolescents (10-20 years old) and 46 % adults (20-60 years old) were among the SCA patients group whereas in the healthy control group, 21 % adolescents (10-20 years old) and 79 % adults (20-60 years old) were present. In both groups, the less represented age groups were those of [0-10] and [50-60] years old.

Results related to the enzymatic antioxidant activities in both groups of participants are presented in Figure 1. The activities of GPX, SOD and CAT significantly increased (between 0.24 and 298.78 UI) among the SCA patients compared to the healthy control (between 0.19 and 212.33 UI).

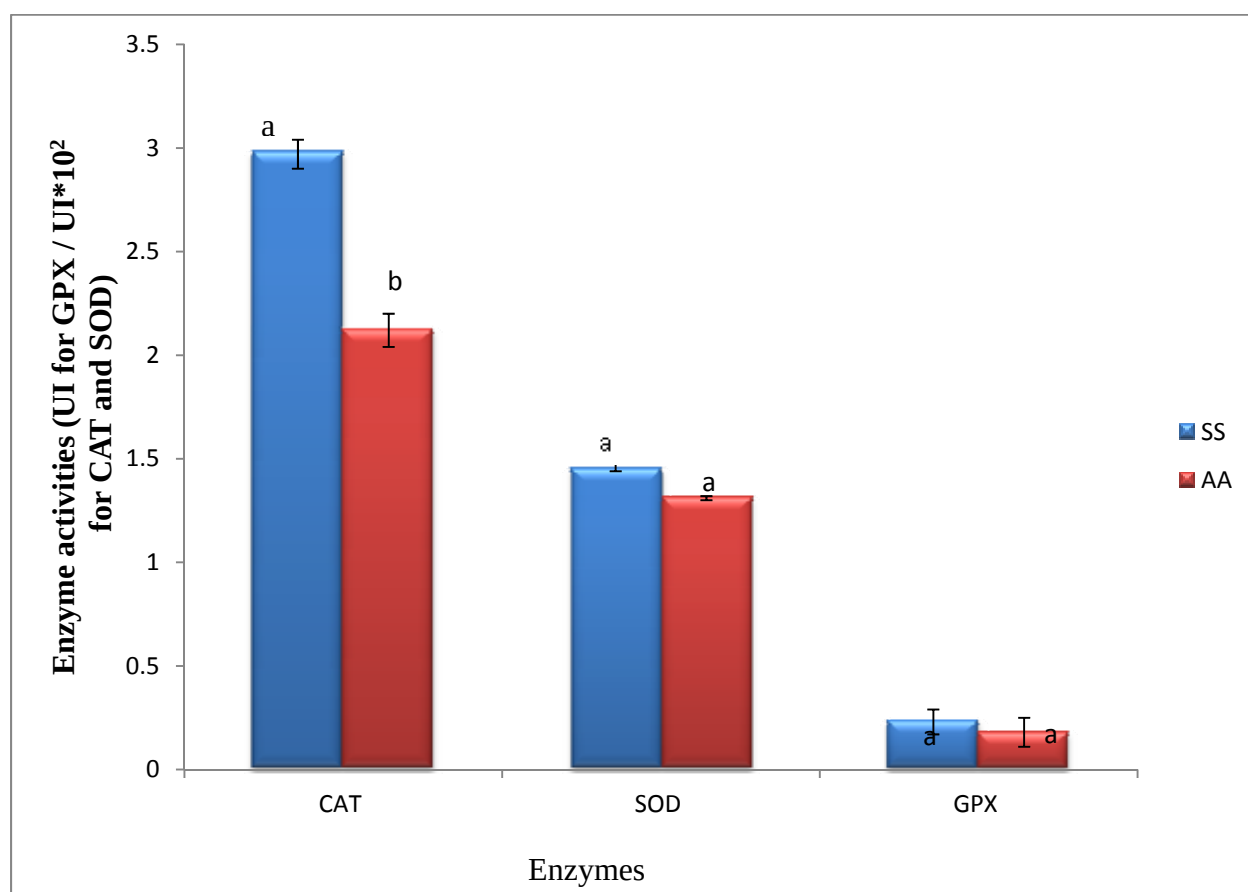


Figure 1: Enzymatic activity values in patients (SS) and control (AA)

Independent t-test. Threshold of significance $P < .05$

This figure presents the average serum activities of the antioxidant enzymes in the form of means $\pm$ standard deviation. SOD (superoxide dismutase); GPX: glutathione peroxidase; CAT: Catalase; Bars with different letters are significantly different ($P < .05$).

MDA, FRAP and reduced Glutathione are indicators of lipid peroxidation and antioxidant capacity whose results are revealed in figure 2. As above results, values of these parameters significantly increased (1.16 ± 0.97 ; 137.74 ± 37.39 and 13.59 ± 9.34 respectively) among the SCA patients compared to healthy control (0.39 ± 0.4 ; 109.44 ± 25.96 and 2.88 ± 1.46).

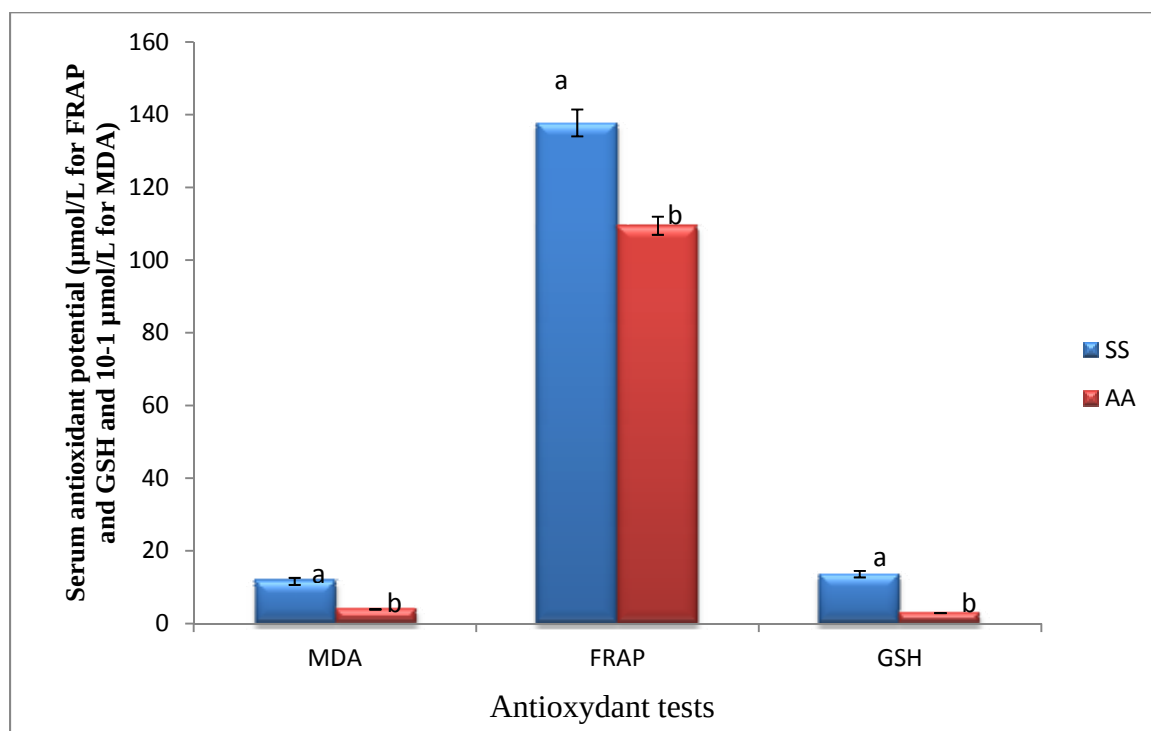


Figure 2: Serum antioxidant potential values in SS and AA

Independent t -test. $P < .05$. This figure presents the serum antioxidant potential in the form of means $\pm$ standard deviation. MDA = malonyldialdehyde; FRAP = ferric reducing antioxidant power; GSH= reduced Glutathione; Bars with different letters are significantly different ($P < .05$).

With regard to the protein-energy status (table 1), the mean values of BMI of sickle cell patients decreased compared to the normal reference range (BMI = 17.95 ± 2.72 ; $< [18.5-25]$) and

significantly reduced (P = 0.000) compared to the healthy control group (25.88±4.73). Albumin and transferrin values are included in the normal range of reference.

Table 1: Protein-energy nutritional status values

Parameters	IMC	Albumin	Transferrin
groups			
Reference values	[18.5-25[	3.4-5.4g/dl	2-3.8g/l
SS	17.95 ± 2.72	3.95 ± 0.51	2.86 ± 1.22
AA	25.88 ± 4.73*	3.55 ± 1.12*	2.48 ± 1.15
P value	.00	.03	.14

Independent t-test. $P < .05$. This table presents the mean values of BMI, albumin and transferrin as mean ± standard deviation. BMI = body mass index, expressed in kg/m^2 ; Albumin expressed in g/dl; Transferrin expressed in g/l. *: significantly different ($P < .05$) in the column.

Table 2 represents the different correlations between oxidative stress parameters and the protein-energetic nutritional status. Pearson correlation revealed a significant association between catalase and BMI (-0.884; 0.000), between reduced glutathione and BMI (-0.889; 0.000) and between MDA and BMI (-0.879; 0.015).

Table 2: Correlation between parameters

CAT	Albumine	Transferrine	IMC
Correlation coefficient	-0.190	-0.106	-0.884*
Sig. (2-tailed)	0.064	0.350	0.000
SOD			
Correlation coefficient	-0.036	-0.048	-0.096
Sig. (2-tailed)	0.738	0.682	0.421
GPX			
Correlation coefficient	0.061	0.176	-0.132
Sig. (2-tailed)	0.594	0.148	0.286
FRAP			
Correlation coefficient	0.022	-0.023	-0.222
Sig. (2-tailed)	0.828	0.837	0.048
MDA			
Correlation coefficient	0.031	-0.077	-0.879*
Sig. (2-tailed)	0.772	0.502	0.015
GSH			
Correlation coefficient	0.155	0.257	-0.889*
Sig. (2-tailed)	0.132	0.021	0.000

161 *Pearson Correlation test. SOD (super oxide dismutase); GPX: glutathione peroxidase; CAT: Catalase. MDA:*
162 *malonyldialdehyde. FRAP: ferric reducing antioxidant power. GSH: reduced glutathione*

163 The effect of some factors on the biochemical parameters studied is included on **table 3** below.
164 Extrinsic and intrinsic factors such **as** age, water consumption, meat and fruit consumption
165 showed some influence (**table 3**). In fact, the GPX activity significantly decreased ($p = 0.030$)
166 with the increase of age and water consumption ($p = 0.089$). Moreover, SOD activity
167 significantly increased**d** ($p = 0.002$) in people who frequently consumed meat and fish. Also, the
168 average values of MDA, FRAP, reduced glutathione and SOD activity are more **or** less although
169 **not** significant, in people who consumed fruits constantly.

170

Table 3: Influence of some intrinsic and extrinsic factors on biochemical parameters in the patients group

Extrinsic and intrinsic factors	Age (year)			water consumption per day (liters)		
	1-20	20-50	P Value	Less than 1.5l	more than 2l	P Value
Albumin	3.95 ± 0.39	3.91 ± 0.62	0.792	4.08 ± 0.463	3.87 ± 0.534	.201
Transferrin	2.81 ± 1.10	2.68 ± 1.12	0.708	2.80 ± 1.348	2.66 ± 1.011	.727
CAT	318.75 ± 95.61	286.09 ± 125.7	0.324	290.44 ± 105.901	310.69 ± 112.981	.555
SOD	141.03 ± 176.1	148.03 ± 137.8	0.891	133.42 ± 119.073	151.04 ± 168.165	.744
GPX	0.33 ± 0.76	0.15 ± 0.75	0.030*	0.35 ± 0.421	0.20 ± 0.131	.089
MDA	1.05 ± 0.99	1.37 ± 0.97	0.285	1.36 ± 1.285	1.16 ± 0.825	.520
FRAP	125.42 ± 38.78	145.52 ± 30.97	0.060	130.55 ± 35.585	138.92 ± 37.416	.464
GSH	15.99 ± 9.23	13.35 ± 9.41	0.349	15.18 ± 10.370	14.22 ± 8.861	.743
Extrinsic factors	Consumption of Meat/fish			Fruit consumption		
	moderate	regular	P value	moderate	regular	P value
Albumin	4.04 ± 0.51	3.85 ± 0.51	0.202	3.93 ± 0.519	3.96 ± 0.525	.891
Transferrin	2.74 ± 1.14	2.68 ± 1.11	0.865	2.65 ± 1.052	2.92 ± 1.405	.550
CAT	316.30 ± 118.61	310.69 ± 112.98	0.451	298.59 ± 96.321	319.00 ± 146.578	.584
SOD	218.40 ± 173.36	291.81 ± 101.92	0.002*	156.13 ± 158.533	104.25 ± 134.043	.400
GPX	0.20 ± 0.13	0.29 ± 0.340	0.264	0.20 ± 0.145	0.37 ± 0.446	.074
MDA	1.14 ± 1.01	1.31 ± 0.99	0.565	1.25 ± 1.119	1.15 ± 0.518	.766
FRAP	137.97 ± 38.70	134.25 ± 35.27	0.732	138.45 ± 32.100	129.15 ± 48.549	.454
GSH	12.74 ± 7.75	16.22 ± 10.42	0.210	15.24 ± 9.642	12.62 ± 8.368	.407

Independent t-test. $P < .05$. This table presents the influence of some intrinsic and extrinsic factors on biochemical parameters. SOD (super oxide dismutase); GPX: glutathione peroxidase; CAT: Catalase. MDA: malonyldialdehyde. FRAP: ferric reducing antioxidant power. GSH: reduced glutathione; *: Significantly different ($P < .05$) between the columns.

DISCUSSION

Sickle cell anaemia (SCA) represents the first genetic disease worldwide [1]. Sickle cell patients simultaneously present a loss of appetite and body composition with an increase of anxiety and stress. Evaluating the relationship between protein-energy status and the oxidative stress state, these could help in the management of sickle cell anaemia.

In this study, it is clearly demonstrated by the average value of BMI that sickle cell patients have a body weight that is significantly lower than that of a healthy individual; reflecting the net impact of sickle cell anaemia (SCA) on the body mass index. That may be due to the growth delay due to the lack of care before the age of 16 years [18]. SCA has a direct impact on the endocrine system in relation to the failure to thrive. According to Olambiwonnu [19, 20], endocrine disturbances are essentially gonadal and adrenal. Moreover, some authors suggest that the level of growth hormone (GH), as well as the response to stimulation under the effect of the factor of hypothalamic release (GHRH) or hypothalamic stimulators are reduced in patients suffering from sickle cell [19]. The consequence could be the synthesis of somatomedins, including type I which is an intermediate hormone helping GH to act on bone maturation, growth of the skeleton and other bodies. In sickle cell patients, chronic anaemia is a limiting factor for physical development, because in case of anaemic crisis and aggravation of hypoxic phenomena. Chronic hypoxia secondary to anaemia and tissue infarction associated with vaso-occlusive events are the main factors triggering the above-mentioned endocrine manifestations. At the peripheral level, kidney failure associated to cell suffering contributes to increase the reduction in synthesis of the somatomedins [21]. On the other hand, a fundamental change of muscle tissue properties could take place in those patients. They have a zinc deficit which is cofactor in the synthesis of collagen [22], which would be detrimental to the development of muscle tissues. The

osteopenia or osteoporosis may also be a cause of the decline in patient's body mass. It is an osteo-articular complication that occurs in sickle cell disease. Some study showed a significant decrease in bone density in sickle cell patients compared to healthy persons [23, 24]. This decrease in bone density will inevitably lead to a reduction in body mass in these patients. All of the above explains the mass weight loss during sickle cell disease. This results also corroborate previous works which also revealed a deficit in body composition in sickle cell patient [25, 26, 27].

Albumin is the most represented protein in the blood used to distribute a number of molecules in the body such as hormones, fatty acids, bilirubin and non water-soluble drugs. In sickle cell patients because of frequent haemolysis, the non conjugate bilirubin remains free in the body. Moreover, patients recruited in this study were under folic acid treatment, a vitamin which is necessary for the maintenance of their blood profile. The increase albumin value in the patients group compared to healthy control group could be explained by the fact that albumin, carrier of free bilirubin and folic acid for example, would have been synthesized in large quantities by the liver.

Transferrin values do not significantly varied in both groups and are included in the normal range of reference. In general, obtaining normal values of transferrin and albumin rates makes difficult the biological diagnosis of protein-energy malnutrition. Nevertheless, BMI level can help in such situation.

The increase of lipid peroxidation may be due to the oxidation of the HbS coupled with the delocalization of iron [28]. Hydrogen peroxide (H_2O_2) is formed in large quantity by ion superoxide (O_2^-), produced during the oxidation of HbS. H_2O_2 in the presence of free iron, produces free radicals which induce to serious damage on the protein and lipid membrane,

235 leading to the oxidation of thiols and lipid peroxidation [28]. The increase in free iron production
236 in relation with a decrease in the synthesis of the hemic group can also act as a Fenton reagent
237 with hydrogen peroxide to produce superoxide, peroxide and hydroxyl radicals. The
238 consequence is the lipid peroxidation, with the increase of MDA values [29]. Many studies have
239 shown that some non-enzymatic antioxidants such as Ascorbic acid, ubiquinone, vitamins A and
240 E, carotene are generally used during oxidative stress while the enzymatic one levels is either
241 increased in case of low oxidative stress, or diminished when the intensity of oxidative stress is
242 too important [30]. Thus, the significant increase in antioxidant enzymes activities observed in
243 this study may reflect the real status of oxidative stress in homozygote SS patients.

244 There is a negative correlation between the parameters of the stress (catalase, MDA and reduced
245 glutathione) and BMI. This result shows that there is a link between oxidative stress and
246 malnutrition which can be related to the decrease in body mass. Indeed, the body composition of
247 humans is made of body fat and lean mass. Thus, a quantitative change in one of these
248 compartments could impact significantly the total body composition [31]. In addition, the body
249 mass organization is made of proteins which can be oxidized to produce free radicals. For
250 example, the alteration of cellular lipids, muscle and skeletal proteins and collagen by free
251 radicals induce a reduction of fat and lean mass leading to the physical disabilities [30].

252 The sport practice and alcohol consumption have no influence on oxidative stress markers
253 because none of the patients in this study was involved in an intense physical activity. It has been
254 demonstrated that intense practice of sports is associated with a risk factor of oxidative stress.
255 During practice of intense physical activity, there is a shortness of breath which leads to a
256 desaturation of the Hb-O₂, and an acceleration of cell sickling [31].

The extrinsic and intrinsic factors influencing the oxidative stress status in this study were feeding, hydration, and age. A good balanced diet (vegetables, fruits, fish, and soybean oil) should theoretically be sufficient to provide antioxidants and micronutrients necessary to minimize harmful effect of ROS [30]. These may justify the reduction in the severity of oxidative stress in patients who frequently consume fruits.

CONCLUSION

The aim of this study was to contribute to a better management of sickle cell anaemia patients by evaluating the relationship between the protein-energy status and the state of oxidative stress. Results suggest that sickle cell patients in Yaoundé-Cameroon are underweight with higher oxidative stress status. Furthermore, they are under stress, they lack a good protein-energy status.

List of abbreviations

FRAP: Ferric Reduction Antioxidant Power

Hb: Haemoglobin

MDA: Malonyldialdehyde

ROS: Reactive Oxygen Species

SCA: Sickle Cell Anaemia

BMI: body mass index

Competing interests

The authors declare that they have no competing interests.

Authors' contributions:

This work was carried out in collaboration between all authors. ‘Author A’ (NYA NKWIKU Prudence Josela) conducted the study and assays, managed the literature searches and wrote the first draft of the manuscript. ‘Author B’ (Biapa Nya Prosper Cabral) designed the research, co-directed the research work as well as the statistical analysis study. ‘Author C’ (Chetcha Bernard) facilitated the contact between principal investigator and SCA patients and helped explaining the importance of such research to patients. ‘Author D’ and F’ (Kengne Fotsing Christian and Yembeu Lena Natacha) assisted in conducting the assays. ‘Author E’ (Pieme Constant Anatole) co-directed the research work. All authors read and approved the final manuscript.

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