

Original Research Article

Protein-energy status and oxidative stress of a group 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 resulted causes the synthesis of abnormal haemoglobin (HbS) which is a pro-oxidant machine induced the 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 anemia patients by evaluating the relationship between protein-energy status and the state of oxidative stress.

Methods: 100 participants have been 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), CAT (catalase) and Superoxide dismutase (SOD) activities using standard methods.

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

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

Conclusion: These results suggested that sickle cell patients in Yaoundé-Cameroon are underweight with higher oxidative stress status. More they are under stress, less 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 anemia (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 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, including 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 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 pro-oxidant machine. These independent events can interact with 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 anemia [8], thus indicating the existence of a protein-energy malnutrition deficiency. A study conducted in Brazzaville (Congo) has proven that children with sickle cell anemia 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 electrolytic profile status on patients suffering of SCA [11] in Yaoundé-Cameroon results 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 for human health of the Center (CRERSH - CE); that gave us an ethical clearance under the reference No. 0283/CRERSHC/2016 on the 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 was informed of the objectives of the study and was requested the written informed consent according to the declaration of Helsinki.

Type and duration and sites of study

We have carried out a joint cross-type case-control study. First of all, a form of questions permitted us to have certain information permitting us to calculate anthropometric parameters (BMI) and to have alimentary habits of persons taking part. 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 in the period of August 15, 2016, to January 15, 2017.

This study was carried out on two different sites, the central hospital of *Yaoundé*, service of Hematology 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, confirmed to be homozygous SCA patients and confirmed to be healthy homozygous individuals (AA), all ages of at least one year. Have not been included in this study, heterozygous individuals (AS), homozygous individuals AA or SS with insufficient kidney disease, inflammatory syndrome or those who have received a blood transfusion of less than 72 h before dialoging.

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 informations 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. Temperature and weight were measured using respectively thermometer and a scale and a fathom allowed measuring the size. Blood collection ensued with the assistance of 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 was evaluated through the determination of 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, we had recourse to the parametric tests. The results were expressed as an average $\pm$ 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 allowed estimating of the level of association between quantitative variables. Data were processed in the Excel software and analyzed with the SPSS software (Statistical Package for Social Sciences) version 16.0. The value $P < .05$ has been considered to be significant.

RESULTS

Out of 100 participants, 27 men (52%) and 25 women (48%) represented SCA patients while 17 men (35%) and 31 women (65%) were among healthy controls. 44% adolescents (10-20 year old) and 46% adults (20-60 year old) were among SCA patient group whereas in healthy controls group, 21% adolescents (10-20 year old) and 79% adults (20-60 year old) were present. In both groups, the less represented age groups was those of [0-10 [and [50-60 [years. Results related to the enzymatic antioxidant activities in both groups of participants are presented in table 1. The activities of Glutathione peroxidase, SOD and catalase significantly increased respectively (between 0.24 and 298.78 UI) among the SCA patients compared to healthy control (between 0.19 and 212.33 UI).

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

Parameters groups	CAT (UI)	SOD (UI)	GPX(UI)
SS	298.78 ± 107.29*	144.89 ± 150.78	0.24 ± 0.25
AA	212.33 ± 81.02*	131.20 ± 117.37	0.18 ± 0.07
P value	0.00	0.63	0.19

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

*This table represents the average serum activities of the antioxidant enzymes in the form of means ± standard deviation. SOD (super oxide dismutase); GPX: glutathione peroxidase; CAT: Catalase; *: significant difference ($P < .05$).*

MDA, FRAP and reduced Glutathione are indicators of lipid peroxidation and antioxydant capacity which results are revealed in table 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)

Table 2: Serum antioxidant potential values in SS and AA

Parameters groups	MDA (µmol / l)	FRAP (µg / mL)	GSH (µmol / l)
SS	1.16 ± 0.97	137.74 ± 37.39	13.59 ± 9.34
AA	$0.39 \pm 0.4^*$	$109.44 \pm 25.96^*$	$2.88 \pm 1.46^*$
P value	.000	.000	.000

Independent *t*-test. $P < .05$. This table represents the serum antioxidant potential in form of means $\pm$ standard deviation. MDA = malonyl dialdehyde; FRAP = ferric reducing antioxidant power; GSH= reduced Glutathione; *: significant difference ($P < .05$) in column.

With regard of protein-energy status (table 3), 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 lower ($P = 0.000$) than healthy control group (25.88 ± 4.73). albumin and transferrin values are included in the normal range of reference.

Table 3 : Protein-energy nutritional status values

groups	Parameters	IMC	Albumin	Transferrin
	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	$25.88 \pm 4.73^*$	$3.55 \pm 1.12^*$	2.48 ± 1.15
	P value	.00	.03	.14

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

Table 4 represents the different correlations between oxidative stress parameters and the proteino-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 4 : Correlation between parameters

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

MDA:

malonedialdehyde. FRAP: ferric reducing antioxidant power. GSH: reduced glutathione

The effect of some factors on the biochemical parameters studied is included on table 5 below. Extrinsic and intrinsic factors such age, water consumption, meat and fruit consumption showed some influence (table 5). In fact, the GPX activity significantly decreased ($p = 0.030$) with the increase of age and water consumption ($p = 0.089$). Moreover, SOD activity significantly increase ($p = 0.002$) in people who frequently consumed meat and fish. Also, the average values of MDA, FRAP, reduced glutathione and SOD activity are more less although no significant, in people who consumed fruits constantly.

182 **Table 5:** influence of some intrinsic and extrinsic factors on biochemical parameters in patient
 183 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

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

DISCUSSION

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

In this study, it is clearly demonstrated by the average value of BMI that sickle cell people have a body weight significantly lower than that of healthy people; reflecting the net impact of sickle cell anemia (SCA) on the body mass index.

That may be due to the growth delay due to the failure to care for 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 intermediate hormone helping GH to act on bone maturation, growth of the skeleton and other bodies. In sickle cell patients, chronic anemia is a limiting factor for physical development, because in case of anemic crisis and aggravation of hypoxic phenomena. Chronic hypoxia secondary to anemia 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 a cofactors in the synthesis of collagen [22], that would be detrimental to the development of muscle tissue. 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 person [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 result 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 patient 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 quantity 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 and leading to the oxidation of thiols and lipid peroxidation [28]. The increase of free iron product in relation with the decrease in the synthesis of the hemic group can also act as a Fenton reagent with hydrogen peroxide to produce superoxide, peroxide and hydroxyl radicals. The consequence is the lipid peroxidation, with the increase of MDA values [29]. Many studies have shown that some non- enzymatic antioxidants such as Ascorbic acid, ubiquinone, vitamins A and E, carotene are generally used during oxidative stress while the enzymatic one levels is either increased in case of low oxidative stress, or diminished when the intensity of oxidative stress is too important [30]. Thus, the significant increase in antioxidant enzymes activities observed in this study may reflects the real status of oxidative stress in homozygote SS patients.

There is a negative correlation between the parameters of the stress (catalase, MDA and glutathione reduced) and BMI this result shows that there is a link between oxidative stress and malnutrition which can be related to the decrease in body mass. Indeed, the body composition of human is made of body fat and lean mass. Thus, a quantitative changes in one of these compartments could impact significantly the total body composition [31]. In addition, body mass organization is made of proteins which can be oxidated and produced free radical. For example, The alteration of cellular lipids, muscle and skeletal proteins and collagen by free radicals induce a reduction of fat and lean mass leading to the physical disabilities [30].

The sport practice and alcohol consumption have no influence on oxidative stress markers because none of the patients in this study was involved in an intense physical activity. It has been demonstrated that intense practice of sport is associated with a risk factor of oxidative stress.

During practice of intense physical activity, there is a shortness of breath which leads to a desaturation of the Hb-O₂, and acceleration of the cell sickling [31].

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

List of abbreviations

FRAP: Ferric Reduction Antioxidant Power

Hb: Haemoglobin

MDA: Malonedialdehyde

ROS: Reactive Oxygen Species

SCA: Sickle Cell Anemia

BMI: body mass index

Competing interests

The authors declare that they have no competing interests.

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