



## RESEARCH ARTICLE

# Relation between haptoglobin polymorphism and oxidative stress status, lipid profile, and cardiovascular risk in sickle cell anemia patients

Christian Bernard Kengne Fotsing<sup>1,2</sup>  | Constant Anatole Pieme<sup>3</sup> | Prosper Cabral Biapa Nya<sup>2</sup>  | Jean Paul Chedjou<sup>4</sup> | Solange Dabou<sup>1</sup> | Carine Nguemeni<sup>5</sup> | Georges Teto<sup>6</sup> | Wilfred Fon Mbacham<sup>4</sup> | Donatien Gatsing<sup>1</sup>

<sup>1</sup>Research Unit of Microbiology and Antimicrobial Substances, Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon

<sup>2</sup>Research Unit of Biochemistry of Medicinal Plants, Food Science and Nutrition, Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon

<sup>3</sup>Laboratory of Biochemistry, Department of Biochemistry and Physiological Science, Faculty of Medicine and Biomedical Science, University of Yaounde I, Yaounde, Cameroon

<sup>4</sup>Laboratory for Public Health Research Biotechnology, Department of Biochemistry, University of Yaounde I, Yaounde, Cameroon

<sup>5</sup>Department of Neurology, University Hospital of Würzburg, Würzburg, Germany

<sup>6</sup>Centre International de Recherche Chantal Biya, Yaounde, Cameroon

## Correspondence

Prosper Cabral Biapa Nya,  
P.O.Box 67 Dschang, Cameroon.  
Email: prbiapa@yahoo.fr

## Funding information

The authors did not receive any funding for this study.

## Abstract

**Objective:** The haptoglobin (Hp) gene located on chromosome 16q22 exhibits a polymorphism that can impact its capacity to inhibit the deleterious oxidative activity of free hemoglobin. We aimed to determine the influence of Hp polymorphism on oxidative stress, lipid profile, and cardiovascular risk in Cameroonian sickle cell anemia patients (SCA patients).

**Method:** The Hp genotypes of 102 SCA patients (SS), 60 healthy individuals (AA), and 55 subjects with sickle cell trait (AS) were determined by allele-specific PCR, and the blood parameters were assessed using standard methods.

**Results:** Hp2-2 genotype was significantly ( $P < .05$ ) present in SS (54%) than in AS (42%) and AA (38%). Levels of catalase and cell reactive protein were higher, while levels of total antioxidant capacity, triglycerides, low-density lipoprotein cholesterol, blood pressure, Framingham score, and body mass index were lower in the SCA patients. These parameters appeared to be unrelated to the haptoglobin genotypes. SCA patients with Hp1-1 genotype presented a higher oxidative stress index ( $0.53 \pm 0.31$ ) than those with Hp2-1 ( $0.33 \pm 0.18$ ). Lipid profile and cardiovascular risk were not significantly different between various Hp genotypes in SCA patients.

**Conclusion:** Haptoglobin polymorphism did not affect lipid profile, cardiovascular risk, and oxidative stress status of SCA patients. Nevertheless, SCA patients with Hp1-1 genotype tended to be more prone to oxidative stress than those with Hp2-1.

## KEYWORDS

Cameroon, haptoglobin, lipid, oxidative stress, polymorphism, sickle cell anemia

## 1 | INTRODUCTION

Haptoglobin (Hp) is an  $\alpha 2$ -sialoglycoprotein that binds free hemoglobin (Hb) and prevents oxidative damage caused by the reaction

between heme iron of Hb and proteins and lipids.<sup>1</sup> The capacity of Hp to neutralize potential oxidation from the heme in Hb is particularly important in sickle cell anemia (SCA), where there is increased release of free Hb and increased systemic oxidative stress due to chronic

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2022 The Authors. *Health Science Reports* published by Wiley Periodicals LLC.

hemolysis. Indeed, SCA is a genetic disease characterized mainly by chronic hemolytic anemia, vaso-occlusive crises (VOCs), and bacterial infections. Chronic hemolysis results in the presence of a large amount of free Hb in the blood. This large amount of free Hb contributes to the formation of reactive oxygen species, thus causing oxidative stress and lipid peroxidation.<sup>2</sup> The oxidative stress can exacerbate atherosclerosis caused by increased accumulation of cholesterol in the arterial wall of macrophages.<sup>3</sup> The presence of free Hb in plasma is also linked to increased endothelial adhesion and nitric oxide depletion,<sup>4</sup> leading to a decrease of its vasodilator and antithrombotic properties.<sup>5</sup> This decrease, in turn, increases the atherogenic risk. The atherogenic risk has long been considered as low in SCA patients because of the reduction of total cholesterol level in plasma, especially during crises. Elsewhere, recent studies have reported disturbances in other markers of the lipid balance, in particular an increase in plasma triglyceride concentrations, ApoB and ApoB/ApoA1, c-LDL/c-HDL, and CT/c-HDL ratios (index of atherogenicity). These lipid abnormalities could facilitate the occurrence of cardiovascular diseases in SCA patients.<sup>6</sup>

Hp protein is present in all mammals, but its polymorphism is particular to humans.<sup>3</sup> Yano et al developed a PCR technique to identify the six different genotypes of Hp (Hp1S-1S, Hp1F-1S, Hp1F-1F, Hp2-1S, Hp2-1F, and Hp2-2).<sup>7</sup> These genotypes do not have equal capacity to protect the body from harmful effects of free Hb.<sup>8</sup> Hp1-1 is more active than the others in binding hemoglobin and suppressing its inflammatory effects.<sup>9</sup> Melamed-Frank et al<sup>10</sup> established that individuals with Hp2-2 are more prone to oxidative stress than others. Hp2-2 individuals show a stronger immunological reactivity than those with Hp1-1 and Hp2-1, as revealed by antibody production after vaccination.<sup>9</sup> Different clinical conditions have been associated with the polymorphism of haptoglobin. Roguin et al<sup>11</sup> showed that Hp2-2 is associated with myocardial infarction, as a predictor of the severity and extent of infarction damage in patients with different risk factors. Ostrowski et al<sup>12</sup> and Moreira and Naoum<sup>13</sup> already reported a strong association between SCA and the Hp1-1 genotype. Other works suggested that Hp polymorphism may be involved in the pathophysiology of SCA.<sup>2,14</sup> More recently, Chintagari et al<sup>15</sup> demonstrated that Hp attenuates the toxic effects of heme and iron released after hemolysis. This functional Hp gene polymorphism may determine the susceptibility to a wide variety of vascular disorders associated with an increase in oxidative stress.<sup>16</sup>

In Cameroon, the prevalence of SCA is between 2% and 3%. The disease affects over 2 million people and causes about 4000 deaths each year. Half of the death occur before the age of 5, making it a real public health problem. Despite these statistics, the modulatory role of the Hp genotype on the oxidative stress, lipid disturbance, and cardiovascular risk in SCA patients is still largely understudied. The present study was therefore designed to investigate the impact of Hp polymorphism on oxidative stress, lipid profile, and atherogenic risk in SCA patients from West Cameroon.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design and subjects

We conducted a cross-sectional descriptive study at the Regional Hospital of Bafoussam (RHB) located in West of Cameroon, from September 2018 to August 2020. One-hundred and two SS patients regularly in consultation in RHB and aged 1-40 years were recruited for this study. Exclusion criteria included pregnancy, and patients with a history of crisis or blood transfusion within the past 3 months. Sixty healthy individuals (AA) and 55 subjects with sickle cell trait (AS) were recruited as controls. Prior to beginning the data collection, a questionnaire collecting clinical history including demographic data with tracing of the major traditional cardiovascular risk factors (age, sex, hypertension, family history of cardiovascular disease, diabetes, and current smoking), acute chest syndrome, priapism, alcohol consumption, disease condition, and treatment history was given to each participant. They were informed about the aims of the study, and written informed consents were obtained from them (or their parents or guardians) according to the Declaration of Helsinki. The study was approved by the Ethics Review and Consultancy Committee (ERCC) of CAMBIN (Cameroon Bioethics Initiative; reference number: CBI/424/ERCC/CAMBIN).

### 2.2 | Methods

#### 2.2.1 | Sample collection and biochemical markers measurements

Ten milliliters of blood was collected in two tubes (5 mL in EDTA tubes and 5 mL in dry tubes). Blood in EDTA tubes was spotted on filter paper for molecular analyses. The blood was sampled from fasting participants and collected by nurses of the RHB. SS, AS, and AA phenotypes were determined by agarose gel electrophoresis using the CELLOGEL kit. Plasmatic total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) concentrations were determined by enzymatic colorimetric assays using an auto analyser (Fully Smart; Biochemical Systems International). Low-density lipoprotein cholesterol (LDL-C) was determined by calculation using the formula of Friedewald:  $LDL-C = TC - [HDL-C + TG/5]$ . Atherogenic index of plasma (AIP) was calculated as the logarithmically transformed ratio of molar concentrations of TG to HDL-C:  $AIP = \log (TG/HDL-C)$ . Blood pressure (BP) was measured with an automatic BP monitor with heart sense (One Plus Healthcare FT-11B) and cardiovascular risk was estimated by calculating the Framingham score (F score).<sup>17</sup> C-reactive protein (CRP) concentration was determined by latex agglutination using a DIALAB kit. For oxidative stress, total antioxidant capacity (TAC) by Ferric Reducing Antioxidant Power assay (FRAP), catalase activity (CAT), malonedialdehyde (MDA), and reduced glutathione (GSH) were evaluated using the methods described by Benzie and Strain,<sup>18</sup> Sinha,<sup>19</sup> Folch et al,<sup>20</sup> and Ellman,<sup>21</sup> respectively. The oxidative stress index (OSI) was calculated as the ratio  $[(MDA/TAC) \times 100]$ . Height and weight were measured using an height gauge and an electronic scale, respectively.

## 2.2.2 | DNA extraction and haptoglobin genotyping

Blood spots on the filter paper were excised with a sterile pair of surgical scissors. DNA was extracted from dried blood spots heated at 100°C in Chelex-100 in buffered Tris-EDTA as previously described by Plowe et al.<sup>22</sup> DNA was stored in the Tris-EDTA buffer at -20°C until Hp genotyping was done by allele-specific PCR, as previously described by Yano et al.<sup>7</sup> Briefly, PCR was performed in a 25-μL reaction mixture

consisting of 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 2.5 mM MgCl<sub>2</sub>, 200 μM of each dNTP, 1.5 U of Taq DNA polymerase, and 0.2 μM of each primer with 1 μL of DNA extract. Cycling conditions were as follows: 95°C for 3 min followed by 35 cycles of 94°C for 40 s, 58°C for 1 min, 72°C for 2 min. Final extension was carried out at 72°C for 5 min. PCR products were stored at 4°C for immediate use or -20°C for long-term use. A T3 thermal cycler (Biometra, UK) was used for the PCR amplification. PCR products were electrophoresed at 50 V for 1 h on 1.2% agarose gel containing ethidium bromide, and the Hp genotypes were determined by observing the DNA fragments under UV light. Table 1 shows the sequences of primers and Table 2 shows the combinations of the primers and predicted sizes of DNA fragments amplified in each reaction.<sup>7</sup>

**TABLE 1** Nucleotide sequences of the primers used

Primer	Oligonucleotide sequence (5'-3')
F3	CAGGAGTATACACCTTAAATG
S2	TTATCCACTGCTTCTCATTG
C42	TTACTGTTAGCGAACC GA
C72	AATTTAAAATTGGCATTTCGCC
C51	GCAATGATGTCACGGATATC

**TABLE 2** Primer set for PCR

Reaction	Primer sets (F-R)	Target alleles	Predicted size (bp)
Reaction 2	F3-C42	Hp <sup>2</sup>	935
Reaction S	C51-S2	Hp <sup>1S</sup>	1200
Reaction F	F3-C72	Hp <sup>1F</sup>	1400

## 2.3 | Data analyses

Data were analyzed using Statistical Package for the Social Sciences 20 (SPSS, IBM). Hp genotype frequencies and individual alleles are presented as percentages. According to the size of a population group, which was above 30, as well as the descriptive explored test of Levene, statistical tests for parametric tests were used. Comparisons were made within and between groups using the Chi-square test associated with z test and one-way ANOVA. Hardy-Weinberg equilibrium (HWE) analyses were carried out using the Plink software. Statistically significant difference was considered at  $P < .05$ .

**TABLE 3** Demographic information

Variables		SS (102)	AS (55)	AA (60)	P value
Gender	Male	52 (51%) <sup>a</sup>	10 (18%) <sup>b</sup>	28 (47%) <sup>a</sup>	<.001*
	Female	50 (49%) <sup>a</sup>	45 (82%) <sup>b</sup>	32 (53%) <sup>a</sup>	
Sex ratio (M/F)		1.04 <sup>a</sup>	0.22 <sup>b</sup>	0.8 <sup>a</sup>	<.001*
Age (y)	Mean ± SD	9.59 ± 7.14 <sup>a</sup>	24.89 ± 16.27 <sup>b</sup>	14.83 ± 7.29 <sup>c</sup>	<.001*
	Min-Max	1-40	1-55	1-36	
Age range (y)	1-18	92 (90%) <sup>a</sup>	23 (42%) <sup>b</sup>	44 (73%) <sup>c</sup>	<.001*
	19-55	10 (10%) <sup>a</sup>	32 (58%) <sup>b</sup>	16 (27%) <sup>c</sup>	
History of cardiovascular stroke	No	100 (98%) <sup>a</sup>	54 (98%) <sup>a</sup>	60 (100%) <sup>a</sup>	.558
	Yes	2 (2%) <sup>a</sup>	1 (2%) <sup>a</sup>	0 (0%) <sup>a</sup>	
History of ACS	No	84 (82%) <sup>a</sup>	54 (98%) <sup>b</sup>	59 (91%) <sup>b</sup>	<.001*
	Yes	18 (18%) <sup>a</sup>	1 (2%) <sup>b</sup>	1 (9%) <sup>b</sup>	
History of diabetes	No	101 (99%) <sup>a</sup>	55 (100%) <sup>a</sup>	60 (100%) <sup>a</sup>	.568
	Yes	1 (1%) <sup>a</sup>	0 (0%) <sup>a</sup>	0 (0%) <sup>a</sup>	
History of priapism**	No	49 (94%) <sup>a</sup>	10 (100%) <sup>a</sup>	28 (100%) <sup>a</sup>	.295
	Yes	3 (6%) <sup>a</sup>	0 (0%) <sup>a</sup>	0 (0%) <sup>a</sup>	
History of PHT	No	102 (100%)	55 (100%)	60 (100%)	
Smoker	No	102 (100%)	55 (100%)	60 (100%)	
Alcohol consumption	No	93 (91%) <sup>a</sup>	31 (56%) <sup>b</sup>	50 (83%) <sup>a</sup>	<.001*
	Yes	9 (9%) <sup>a</sup>	24 (44%) <sup>b</sup>	10 (17%) <sup>a</sup>	

Note: Values subscripted with different letters in the same line are significantly different at  $P < .05$ .

Abbreviations: ACS, acute chest syndrome; PHT, pulmonary hypertension.

\*Significant difference at  $P < .05$ . \*\*Done only for male.

## 3 | RESULTS

Table 3 shows characteristics of the study population. Ninety percent of SS patients were children (age range 1-18) with a sex ratio (male/

female) of 1.04. Significant difference was found between the cases (SS) and controls (AA) in age ( $9.59 \pm 7.14$  vs  $14.83 \pm 7.29$ ) and history of acute chest syndrome (ACS) (Yes: 18% vs 9%). Significant difference was found between SS and AS in age ( $9.59 \pm 7.14$  vs  $24.89$

Parameters	SS (n = 102)	AS (n = 55)	AA (n = 60)	P value
TAC ( $\mu\text{M}$ )	$129 \pm 25^b$	$144 \pm 25^a$	$147 \pm 19^a$	<.001*
MDA ( $\mu\text{M}$ )	$0.51 \pm 0.29$	$0.44 \pm 0.34$	$0.54 \pm 0.25$	.183
GSH ( $\mu\text{M}$ )	$29.28 \pm 4.97^b$	$26.39 \pm 5.23^c$	$33.87 \pm 3.66^a$	<.001*
CAT (IU/min/mg)	$780 \pm 233^a$	$490 \pm 196^b$	$477 \pm 112^b$	<.001*
OSI	$0.43 \pm 0.29^a$	$0.31 \pm 0.22^b$	$0.38 \pm 0.22^{a,b}$	.022*
TG (g/L)	$1.31 \pm 0.57$	$1.10 \pm 0.54$	$1.28 \pm 0.53$	.070
TC (g/L)	$1.07 \pm 0.38^b$	$1.39 \pm 0.45^a$	$1.37 \pm 0.39^a$	<.001*
HDL-C (g/L)	$0.43 \pm 0.15$	$0.45 \pm 0.13$	$0.45 \pm 0.10$	.735
LDL-C (g/L)	$0.37 \pm 0.31^b$	$0.72 \pm 0.43^a$	$0.66 \pm 0.44^a$	<.001*
AIP	$0.47 \pm 0.21^a$	$0.38 \pm 0.23^b$	$0.43 \pm 0.17^a$	.036*
SBP (mmHg)	$91.8 \pm 18.1^b$	$117.9 \pm 26.4^a$	$116.4 \pm 13.4^a$	<.001*
DBP (mmHg)	$47.2 \pm 15.1^b$	$74.1 \pm 22.1^a$	$70.5 \pm 10.9^a$	<.001*
F score	$-7.69 \pm 4.58^b$	$-5.13 \pm 6.63^a$	$-6.90 \pm 5.08^b$	.017*
BMI ( $\text{m}/\text{kg}^2$ )	$16.88 \pm 2.69^c$	$24.98 \pm 7.80^a$	$20.32 \pm 3.84^b$	<.001*
CRP (mg/L)	$19.7 \pm 18.9^a$	$8.2 \pm 5.5^b$	$6.0 \pm 0.0^b$	.046*

Note: Values subscripted with different letters in the same line are significantly different at  $P < .05$ ; ANOVA test with post hoc Dunnett T3.

Abbreviation: AIP, arterogenic index of plasma ( $\text{AIP} = \log(\text{TG}/\text{HDL-C})$ ); BMI, body mass index; CAT, specific activity of catalase ( $\mu\text{mol}/\text{L}$  of  $\text{H}_2\text{O}_2/\text{min}/\text{mg}$  of protein); CRP, C-reactive protein; DBP, diastolic blood pressure; F score, Framingham score; MDA, malondialdehyde; OSI, oxidative stress index ( $\text{OSI} = (\text{MDA}/\text{TAC}) \times 100$ ); SBP, systolic blood pressure; TAC, total antioxidant capacity.

\*Significant difference at  $P < .05$ .

TABLE 4 Parameters measured in the studied groups

TABLE 5 Haptoglobin genotypes and allele frequencies distribution in the study population

Groups		SS (n = 102)	AS (n = 55)**	AA (n = 60)**	P value	Total
Hp genotypes	Hp 1-1	32 (31.4%) <sup>a</sup>	16 (29.1%) <sup>a</sup>	21 (35%) <sup>a</sup>	<.001*	69 (32%)
	Hp 2-1	15 (14.7%) <sup>b</sup>	23 (41.8%) <sup>a</sup>	23 (38.3%) <sup>a</sup>		61 (28%)
	Hp 2-2	55 (53.9%) <sup>a</sup>	16 (29.1%) <sup>b</sup>	16 (26.7%) <sup>b</sup>		87 (40%)
Hp subtypes	Hp1S-1S	25 (24.5%) <sup>a</sup>	8 (14.5%) <sup>a</sup>	9 (15%) <sup>a</sup>	.001*	42 (19%)
	Hp1F-1F	6 (5.9%) <sup>a</sup>	6 (10.9%) <sup>a</sup>	9 (15%) <sup>a</sup>		21 (10%)
	Hp1S-1F	1 (1%) <sup>a</sup>	2 (3.6%) <sup>a</sup>	3 (5%) <sup>a</sup>		6 (3%)
	Hp2-1S	11 (10.8%) <sup>a</sup>	13 (23.6%) <sup>a</sup>	13 (21.7%) <sup>a</sup>		37 (17%)
	Hp2-1F	4 (3.9%) <sup>b</sup>	10 (18.2%) <sup>a</sup>	10 (16.7%) <sup>a</sup>		24 (11%)
	Hp2-2	55 (53.9%) <sup>a</sup>	16 (29.1%) <sup>b</sup>	16 (26.7%) <sup>b</sup>		87 (40%)
Hp allele frequencies	Hp <sup>1S</sup>	0.31	0.28	0.28		0.29
	Hp <sup>1F</sup>	0.08	0.22	0.26		0.17
	Hp <sup>2</sup>	0.61	0.50	0.46		0.54
	Hp <sup>1</sup>	0.39	0.50	0.54		0.46
	OR	0.54	0.85	1.87		
	95% CI	0.34-0.84	0.50-1.42	1.19-2.95		

Note: a-c: values subscripted with different letters in the same line are significantly different at  $P < .05$ .

\*Chi<sup>2</sup> test with Bonferroni adjustment and Fisher test for the small size groups.

\*\*AS and AA were in Hardy-Weinberg equilibrium ( $P > .05$ ).

**TABLE 6** Oxidative stress parameters, lipid profile, artherogenic index of plasma, blood pressure, Framingham score, and body mass index according to Hp genotype among the studied groups

Grp	Hp	TAC ( $\mu\text{M}$ )	MDA ( $\mu\text{M}$ )	GSH ( $\mu\text{M}$ )	CAT (U/min/mg)	OSI	TG (g/L)	TC (g/L)	HDL-C (g/L)	LDL-C (g/L)	AIP	SBP (mmHg)	DBP (mmHg)	F score	BMI ( $\text{m}/\text{kg}^2$ )
SS	Hp 1-1	125 ± 27	0.61 ± 0.28	28.2 ± 4.7	779 ± 272	0.53 ± 0.31 <sup>a</sup>	1.5 ± 0.7	1.1 ± 0.5	0.46 ± 0.22	0.36 ± 0.35	0.52 ± 0.23	96 ± 19	51 ± 16	-8.7 ± 4.8	17 ± 3
	Hp 2-2	130 ± 24	0.48 ± 0.31	29.7 ± 4.8	770 ± 215	0.39 ± 0.28 <sup>ab</sup>	1.2 ± 0.4	1.0 ± 0.3	0.42 ± 0.11	0.38 ± 0.31	0.45 ± 0.20	90 ± 18	46 ± 15	-6.8 ± 4.5	16 ± 2
	Hp 2-1	132 ± 26	0.42 ± 0.21	30.2 ± 5.9	822 ± 220	0.33 ± 0.18 <sup>b</sup>	1.3 ± 0.6	1.0 ± 0.3	0.44 ± 0.11	0.34 ± 0.25	0.43 ± 0.18	90 ± 11	42 ± 11	-8.9 ± 3.9	16 ± 2
	P value	.509	.072	.308	.742	< .05*	.053	.631	.546	.897	.271	.315	.237	.106	.559
AS	Hp 1-1	143 ± 20	0.54 ± 0.55	28.9 ± 5.5 <sup>a</sup>	506 ± 186	0.38 ± 0.35	1.1 ± 0.7	1.4 ± 0.3	0.43 ± 0.11	0.71 ± 0.33	0.38 ± 0.25	118 ± 28	74 ± 22	-3.4 ± 7.2	23 ± 6
	Hp 2-2	136 ± 38	0.43 ± 0.21	24.5 ± 4.0 <sup>b</sup>	480 ± 226	0.31 ± 0.14	1.2 ± 0.5	1.4 ± 0.5	0.48 ± 0.13	0.71 ± 0.52	0.41 ± 0.22	121 ± 26	73 ± 16	-5.8 ± 6.8	25 ± 9
	Hp 2-1	149 ± 13	0.38 ± 0.20	26.0 ± 5.3 <sup>ab</sup>	486 ± 189	0.25 ± 0.12	1.0 ± 0.5	1.4 ± 0.5	0.44 ± 0.14	0.74 ± 0.46	0.35 ± 0.22	116 ± 27	75 ± 26	-6 ± 6.01	26 ± 9
	P value	.288	.349	< .05*	.930	.236	.458	.890	.558	.958	.782	.875	.983	.410	.645
AA	Hp 1-1	146 ± 17 <sup>ab</sup>	0.51 ± 0.24	34.3 ± 3.8	457 ± 137	0.36 ± 0.16	1.2 ± 0.5	1.4 ± 0.4	0.45 ± 0.09	0.68 ± 0.45	0.40 ± 0.17	120 ± 12	75 ± 12	-6.1 ± 4.6	21 ± 3.4
	Hp 2-2	136 ± 27 <sup>b</sup>	0.61 ± 0.29	32.6 ± 4.1	516 ± 108	0.49 ± 0.32	1.3 ± 0.6	1.5 ± 0.4	0.44 ± 0.08	0.76 ± 0.44	0.43 ± 0.16	116 ± 13	66 ± 9	-6.8 ± 5.7	19 ± 3.7
	Hp 2-1	156 ± 9 <sup>a</sup>	0.53 ± 0.24	34.4 ± 3.2	468 ± 84	0.34 ± 0.14	1.3 ± 0.5	1.3 ± 0.4	0.45 ± 0.11	0.58 ± 0.42	0.45 ± 0.19	113 ± 15	70 ± 11	-7.7 ± 5.1	21 ± 4.1
	P value	< .05*	.512	.299	.250	.090	.758	.444	.957	.448	.707	.595	.284	.566	.321

Note: Values subscripted with different letters in the same column and in the same group are significantly different at  $P < .05$ ;

\*significant difference at  $P < .005$ ; ANOVA test; Bold values are significant at  $p < .05$ .

Abbreviations: AIP, artherogenic index of plasma ( $\text{AIP} = \log(\text{TG}/\text{HDL-C})$ ); BMI, body mass index; CAT, specific activity of catalase ( $\mu\text{mol}/\text{L}$  of  $\text{H}_2\text{O}_2/\text{min}/\text{mg}$  of protein); DBP, diastolic blood pressure; F score, Framingham score; Grp. groups; MDA, malondialdehyde; OSI, oxidative stress index ( $\text{OSI} = [\text{MDA} / \text{TAC}]^{100}$ ); SBP, systolic blood pressure; TAC, total antioxidant capacity.

$\pm 16.27$ ), sex (male: 51% vs 18%), history of ACS (yes: 18% vs 2%), and alcohol consumption (yes: 9% vs 44%).

Oxidative stress parameters, lipid profile, blood pressure, *F* score, and body mass index among the studied groups, irrespective to the Hp genotype, are shown in Table 4. Homozygous SS patients exhibited significantly higher ( $P < .05$ ) activity of CAT ( $780 \pm 233$  IU/min/mg) and amount of CRP ( $19.7 \pm 18.9$  mg/L) and significantly lower ( $P < .05$ ) amounts of TAC ( $129 \pm 25$   $\mu$ M), GSH ( $29.28 \pm 4.97$   $\mu$ M), TC ( $1.07 \pm 0.38$  g/L), LDL-C ( $0.37 \pm 0.31$  g/L), SBP ( $91.8 \pm 18.1$  mmHg), DBP ( $47.2 \pm 15.1$  mmHg), and BMI ( $16.88 \pm 2.69$  m/kg<sup>2</sup>) than AA controls. Compared with AS, SS patients presented significantly ( $P < .05$ ) higher levels of GSH ( $26.39 \pm 5.23$  vs  $29.28 \pm 4.97$   $\mu$ M), CAT activity ( $490 \pm 196$  vs  $780 \pm 233$  IU/min/mg), OSI ( $0.31 \pm 0.22$  vs  $0.43 \pm 0.29$ ), and AIP ( $0.38 \pm 0.23$  vs  $0.47 \pm 0.21$ ), but significantly lower ( $P < .05$ ) amounts of TAC ( $144 \pm 25$  vs  $129 \pm 25$   $\mu$ M), TC ( $1.39 \pm 0.45$  vs  $1.07 \pm 0.38$  g/L), LDL-C ( $0.72 \pm 0.43$  vs  $0.37 \pm 0.31$  g/L), SBP ( $117.9 \pm 26.4$  vs  $91.8 \pm 18.1$  mmHg), DBP ( $74.1 \pm 22.1$  vs  $47.2 \pm 15.1$  mmHg), *F* score ( $-5.13 \pm 6.63$  vs  $-7.69 \pm 4.58$ ), and BMI ( $24.98 \pm 7.80$  vs  $16.88 \pm 2.69$  m/kg<sup>2</sup>).

Table 5 shows the Hp genotype and allele frequency distributions in the study population. The genotype distributions were in HWE in AS ( $\chi^2 = 0.22$ ,  $P = .28$ ) and controls AA ( $\chi^2 = 0.08$ ,  $P = .08$ ). Significant deviation from the Hardy-Weinberg equilibrium was observed in the cases SS ( $\chi^2 = 3.14 \times 10^{-12}$ ,  $P = 9.42 \times 10^{-13}$ ), with heterozygous deficit. Hp2-2 genotype was significantly ( $P < .05$ ) present in homozygous SS patients (54%) than in AS and AA, while Hp2-1 was most significantly found ( $P < .05$ ) in AS (42%) and AA (38%). Hp2-1F was significantly more present in AS and AA than in SS. Regarding intra-group Hp allele frequency distribution, Hp<sup>2</sup> was the most present allele in SS with a proportion of 0.61. The proportion of Hp<sup>1</sup> was 0.38 (with 0.31 for Hp<sup>15</sup> and only 0.08 for Hp<sup>1F</sup>). However, in AS and AA controls, the proportions of the Hp<sup>1</sup> and Hp<sup>2</sup> alleles were similar (around 0.5 each) (Table 5).

Table 6 shows the quantification of different parameters according to Hp genotype in the study population. SS patients with Hp1-1 genotype presented a significantly high ( $P = .043$ ) OSI compared with Hp2-1 patients ( $0.53 \pm 0.31$  vs  $0.33 \pm 0.18$ ). Apart for OSI, no significant difference was observed in SS patients. In AS, GSH was significantly higher ( $P = .048$ ) in Hp1-1 subjects ( $28.9 \pm 5.5$   $\mu$ M) than those with Hp2-2 genotype ( $24.5 \pm 4.0$   $\mu$ M), while in AA controls, TAC was significantly higher ( $P = .007$ ) in Hp2-1 persons ( $156 \pm 9$   $\mu$ M) than those with Hp2-2 genotype ( $136 \pm 27$   $\mu$ M).

## 4 | DISCUSSION

The present study showed a sex ratio (M/F) of 1.04 in SS patients, underlining that SCA distribution is independent of the sex. This can be justified by the recessive autosomic transmission of SCA. The majority of patients were young (mean age: 9.5 years) with 92% of SCA patients aged below 19 years. The early mortality of SCA patients might explain this result. Indeed, according to Houwing et al.,<sup>23</sup> more than half of SCA patients die before the age of 5 years in sub-Saharan Africa. These results are in agreement with those of

Fatima et al.,<sup>24</sup> who found that the most affected by SCA were children under 10 years. The significant difference found in the history of ACS between SS, AS, and AA could be the result of the disease, as ACS is a common complication in SCA.<sup>25</sup>

In the present study, homozygous SS patients exhibited a significant reduction in the TAC level compared with AA and AS, supporting previous studies.<sup>26-28</sup> TAC reflects the reducing property of non-protein individual antioxidant, and this value is more informative than the quantification of individual antioxidants.<sup>26</sup> His increase could be the result of the excess of reactive oxygen species (ROS) in blood. We also noted a significant reduction of GSH level in SS compared with AA, supporting previous studies.<sup>29-31</sup> GSH is another important non-protein low-molecular-weight compound implicated in the fight against oxidative stress.<sup>32</sup> This compound is an essential cofactor for GSH-Px activity. It has been reported that GSH concentration was decreased in SCA patients due to the excessive production of ROS, which consume GSH, leading to the reduction in the activity of GSH-Px.<sup>31</sup> Various studies have shown the depleted levels of nonenzymatic antioxidant molecules such as vitamin C, carotene, and trace elements in SCA patients, reducing antioxidant activity.<sup>33,34</sup> Reduction of TAC and GSH could be the result of higher oxidative stress in SCA patients. Indeed, SCA patients were more affected by the oxidative stress than AS and controls, supported by the OSI, which is higher in SCA patients than AS and AA. Significant increase in the catalase activity was observed in SCA patients compared with controls and AS, supporting the previous study of Faes et al.<sup>35</sup> CAT is an enzyme produced to fight against free radicals, and the increase of its activity is consistent with higher oxidative stress in SCA.<sup>27</sup> It is usually accepted that oxidative stress is increased in SCA compared with healthy conditions. High oxidative stress in SCA may be due to several mechanisms including (a) the excessive levels of cell-free hemoglobin with its catalytic action on oxidative reactions, (b) the characteristic recurrent ischemia-reperfusion injury, (c) a chronic pro-inflammatory state, and (d) higher auto-oxidation of HbS.<sup>36</sup>

SCA patients presented higher CRP level compared with AS and AA controls. The increase in CRP level could be due to infections. Indeed, SCA is mainly characterized by higher infection sensitivity and CRP level increase in case of infection. They also exhibited a significantly lower amount of TC, LDL-C, SBP, DBP, and BMI compared with AS and AA controls. Decreased TC and LDL-C in SCA have been well documented.<sup>3,6,37,38</sup> Hypocholesterolemia in SCA patients might result from the increase in cholesterol use during the intense activity of erythropoiesis. TC, in particular LDL-C, has a well-established role in atherosclerosis. Reduction in TC, HDL-C, and LDL-C can be induced by hemolytic stress in SCA.<sup>39</sup> The low levels of SBP, DBP, BMI, AIP, and LDL-C in SCA patients are consistent with the low levels of TC and *F* score and the virtual absence of atherosclerosis among SCA patients.<sup>3</sup> Previous studies on TG in SCA have given controversial results. Increased TG has been seen in several studies of adult SCA patients.<sup>40,41</sup> However, Shores et al.<sup>42</sup> did not find increased TG levels in adult SCA patients, and Hama et al.<sup>39</sup> found a decrease TG in young SCA patients compared with controls in Iraq. Potential reasons for inconsistencies in TG between studies include differences in age, gender, weight, diet, smoking, disease severity, and treatments.<sup>3</sup> In the



present study, TG level in SCA patients was not significantly different from those of AA and AS. SS patients also exhibited significantly higher ( $P < .05$ ) levels of GSH, OSI, and AIP and significantly lower ( $P < .05$ )  $F$  score compared with AS. The differences in GSH and OSI are the result of higher oxidative stress in SCA. The variation in AIP could be the result of age and sex differences between the two groups, as the mean age of SS individuals ( $9.59 \pm 7.14$ ) is significantly lower than in AS ( $24.89 \pm 16.27$ ) and the proportion of SS females (49%) is significantly lower than in AS (82%).

In the current study, distribution of Hp genotypes was in HWE in AS ( $\chi^2 = 0.22, P = .28$ ) and controls AA ( $\chi^2 = 0.08, P = .08$ ), but a significant deviation from HWE was observed in SS ( $\chi^2 = 3.14 \times 10^{-12}, P = 9.42 \times 10^{-13}$ ), with heterozygous deficit, supporting the previous work of Ostrowski et al<sup>12</sup> where control AA cases were in HWE while SCA patients showed deviation from HWE. The deviation from HWE observed in SS patients may be explained by a lack of random reproduction (panmixia) in this group. Indeed, SCA patients aware of their genetic status tend to choose partners of the AA genotype in order to limit the risk of transmission to children. The Hp2-2 genotype was the prevalent genotype in SCA patients (54%), whereas Hp2-1 was significantly more represented in AS and AA (42% and 38%, respectively). Regarding the allele frequency distribution, Hp<sup>2</sup> was the most frequent in SS patient (0.62). These results support those obtained by Adekile and Haider<sup>2</sup> in Kuwaiti patients. Their research showed that the most frequent genotypes were Hp2-2 (52%) in SS patients and Hp2-1 (49%) in AA controls, and the frequency of Hp<sup>2</sup> allele in SS patient was 0.74. However, these are different from the results of Olatunya et al<sup>43</sup> in Nigeria, Khalid and Khalil<sup>44</sup> in Sudan, Moreira and Naoum<sup>13</sup> in Brazil, and Ostrowski et al<sup>12</sup> in the United States, where the most frequent Hp genotype in SS patients was Hp1-1 (43%, 68%, 36% and 72%, respectively). Furthermore, the previous work of Bruna et al<sup>45</sup> in Brazil showed Hp2-1 as the most frequent Hp genotype in SS patients (57%). The outcome of the relationship between Hp genotypes and SCA may be influenced by a multitude of disease determinants including ethnicity, environmental factors, and other diseases like malaria. SCA patients are commonly affected by malaria, as Cameroon is an endemic region of malaria. Hp has a protective effect against malaria, and it has been suggested that Hp2-2 genotype accelerates the acquisition of immunity against malaria.<sup>46</sup> Previous studies have demonstrated that Hp2-2 genotype is associated with reduced susceptibility to malaria. In the study by Elagib et al<sup>47</sup> in Ghana, they found that Hpl-I genotype was associated with susceptibility to falciparum malaria and the development of severe complications. Quaye et al<sup>48</sup> found that Hp2-2 genotype was significantly less in malaria patients as well as in complications of malaria disease in Sudan. Atkinson et al<sup>49</sup> conducted a similar study in Kenya, with results suggesting that the Hp2-2 genotype was associated with reduced episodes of clinical malaria. These results can explain why Hp2-2 genotype is significantly more represented in SCA patients.

The analysis of the relation between Hp genotyping and lipid profile, oxidative stress parameters, atherogenic index of plasma, BP,  $F$  score, and body mass index in the studied groups revealed significant differences for OSI in SS, for TAC in AA controls, and for GSH in AS. To the best of our knowledge, our study is the first to examine

the relationship between Hp polymorphism and oxidative stress, lipid profile, and cardiovascular risk among Cameroonian SCA patients. In SS, patients with Hp1-1 genotype presented a significantly high ( $P < .05$ ) OSI compared with Hp2-1 patients, underlining the higher susceptibility of Hp1-1 SCA patients to oxidative stress compared with Hp2-1 patients. This result is different from those of Melamed-Frank et al,<sup>10</sup> who established that individuals with Hp2-2 are more prone to oxidative stress than Hp1-1 and Hp2-1 individuals. This difference can be attributed to the difference in experimental approaches. The work of Melamed-Frank et al was performed in vitro using a recombinant truncated Hp, while our study was done on SCA patients. The increase OSI in Hp1-1 SCA patients could be the result of the observed higher level of MDA in these patients compared with Hp2-1 SCA patients ( $0.61 \pm 0.28$  vs  $0.42 \pm 0.21 \mu\text{M}$ ). The other parameters (lipid profile, MDA, TAC, CAT, GSH, atherogenic index of plasma, blood pressure,  $F$  score, and body mass index) did not reveal any significant difference between Hp genotypes and in patients and controls. These results are similar to those of some previous works concerning the effect of Hp genotype on some biochemical parameters in SCA patients. Cox et al<sup>50</sup> showed that Hp polymorphism did not have any effect on the risk of transcranial Doppler among SCA patients in Tanzania; Bruna et al<sup>45</sup> found no relationship between Hp polymorphism and IL-6 and IL-8 levels among Brazilian SCA patients; and Olatunya et al<sup>43</sup> showed that there were no statistical differences in the clinical events and laboratory parameters regarding Hp genotypes in Nigerian SCA patients. Yet, several studies have shown that Hp polymorphism is associated with abnormalities in others diseases. Hp2-2 is characterized by a higher risk of mortality in tuberculosis and HIV.<sup>51</sup> It is a major risk factor for (a) vascular complications and retinopathy in diabetics,<sup>52</sup> (b) cardiovascular disease in diabetes mellitus,<sup>53</sup> (c) developing refractory hypertension in hypertensive patients, (d) severity and extent of myocardial damage after myocardial infarction,<sup>54</sup> (e) the prediction of atherosclerosis in patients with ischemic stroke,<sup>55</sup> (f) non-muscle invasive bladder cancer incidence and progression,<sup>56</sup> and (g) worse glycemic and insulinemic compensation in pediatric obesity.<sup>57</sup> In the present study, TAC and GSH levels were significantly lower in Hp2-2 subjects (AA and AS) than in those carrying Hp1-1 and Hp2-1. This result supports the fact that Hp2-2 is a risk factor for the development of oxidative stress in normal condition. The impaired ability of the Hp2-2 protein to prevent Hb-driven oxidation as compared with the Hp1-1 protein might constitute the mechanism by which oxidative stress and vascular risk in Hp2-2 individuals are increased.<sup>1</sup> Individuals with the Hp2-2 genotype have reduced clearance of the macrophage-Hp-Hb complex, which affects iron deposition, oxidative stress, and active macrophage accumulation. These changes would be consistent with an increased risk of atherosclerotic cardiovascular diseases.<sup>16</sup> Therefore, the mechanism by which SCA patients with Hp1-1 are more prone to oxidative stress than those with Hp2-1 genotype should be investigated. Since a large body of evidence suggests that the Hp<sup>2</sup> allele is a major susceptibility gene for the development of vascular complications and of oxidative stress, a special focus should be given to the particular population of SS patients with Hp2-2 genotype.

## 5 | CONCLUSION

This study aimed to determine the influence of Hp polymorphism on oxidative stress, lipid profile, and cardiovascular risk in Cameroonian SCA patients. Our results showed that although levels of CAT and CRP were higher and levels of TAC, TC, LDL-C, SBP, DBP, F score, and BMI were lower in SCA patients, they appeared not to be related to the haptoglobin genotypes. Hp gene polymorphism did not affect lipid profile, cardiovascular risk, and oxidative stress status of SCA patients. Nevertheless, SCA patients with the Hp1-1 genotype present a higher oxidative stress index than those with Hp2-1. Further investigations are necessary to identify the implications of Hp polymorphism in SCA patients.

### ACKNOWLEDGEMENT

We thank Mr KEPTCHEU Désiré and the staff of the Laboratory of Regional Hospital of Bafoussam for their advice and practical support during sample collection, and Mr TAH Calvino and Mr NANA William of the Laboratory for Public Health Research Biotechnology of Nkolbisson for their help during Hp genotyping.

### CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

### AUTHOR CONTRIBUTIONS

Conceptualization: Christian Bernard Kengne Fotsing, Prosper Biapa Nya, Donatien Gatsing.

Data curation: Christian Bernard Kengne Fotsing, Prosper Biapa Nya.

Formal analysis: Christian Bernard Kengne Fotsing, Solange Dabou, Prosper Biapa Nya.

Investigation: Christian Bernard Kengne Fotsing, Solange Dabou.

Methodology: Christian Bernard Kengne Fotsing, Jean Paul Chedjou, Georges Teto, Carine Nguemeni, Constant Anatole Pieme, Wilfred Fon Mbacham.

Project administration: Prosper Biapa Nya, Donatien Gatsing.

Supervision: Donatien Gatsing.

Writing-Original Draft Preparation: Christian Bernard Kengne Fotsing.

Writing- Review and Editing: Christian Bernard Kengne Fotsing, Prosper Biapa Nya, Jean Paul Chedjou, Solange Dabou, Carine Nguemeni, Georges Teto, Constant Anatole Pieme, Wilfred Fon Mbacham, Donatien Gatsing.

All authors have read and approved the final version of the manuscript.

Prosper Biapa Nya had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

### TRANSPARENCY STATEMENT

Prosper Cabral Biapa Nya affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

### ETHICAL STATEMENT

The study was approved by the Ethics Review and Consultancy Committee (ERCC) of CAMBIN (Cameroon Bioethics Initiative) with reference no: CBI/424/ERCC/CAMBIN. Furthermore, written informed consent of participants/parents/caregivers was obtained.

### ORCID

Christian Bernard Kengne Fotsing  <https://orcid.org/0000-0002-4837-5819>

Prosper Cabral Biapa Nya  <https://orcid.org/0000-0003-4830-9966>

### REFERENCES

1. Goldenstein H, Levy N, Ward J, Costacou T, Levy A. Haptoglobin genotype is a determinant of haemoglobin adducts and vitamin E content in HDL. *J Diabetes Res*. 2018;2018:6125420.
2. Adekile A, Haider M. Haptoglobin gene polymorphisms in sickle cell disease patients with different S-globin gene haplotypes. *Med Princ Pract*. 2010;19:447-450.
3. Zorca S, Freeman L, Hildesheim M, et al. Lipid levels in sickle-cell disease associated with haemolytic severity, vascular dysfunction and pulmonary hypertension. *Br J Haematol*. 2010;149:436-445.
4. Schaer DJ, Buehler PW, Alayash AI, Belcher JD, Vercellotti GM. Hemolysis and free hemoglobin revisited: exploring haemoglobin and heme scavengers as a novel class of therapeutic proteins. *Blood*. 2013;121(8):1276-1284.
5. Graw JA, Yu B, Rezoagli E, et al. Endothelial dysfunction inhibits the ability of haptoglobin to prevent hemoglobin-induced hypertension. *Am J Physiol Heart Circ Physiol*. 2017;312(6):H1120-H1127.
6. Gueye TF, Ndour EH, Cissé F, et al. Perturbations de paramètres lipidiques au cours de la drépanocytose. *Rev Cames Sante*. 2014;2(2):35-41.
7. Yano A, Yamamoto Y, Miyaishi S, Ishizu H. Haptoglobin genotyping by allele specific polymerase chain reaction amplification. *Acta Med Okayama*. 1998;52(4):173-181.
8. Bamm V, Geist A, Harauz G. Correlation of geographic distributions of haptoglobin alleles with prevalence of multiple sclerosis (MS)-a narrative literature review. *Metab Brain Dis*. 2017;32:19-34.
9. Langlois M, Delanghe J. Biological and clinical significance of haptoglobin polymorphism in humans. *Clin Chem*. 1996;42(10):1589-1600.
10. Melamed-Frank M, Lache O, Enav B, et al. Structure-function analysis of the antioxidant properties of haptoglobin. *Blood*. 2001;98(13):3693-3698.
11. Roguin A, Hochberg I, Nikolsky E, et al. Haptoglobin phenotype as a predictor of restenosis after percutaneous transluminal coronary angioplasty. *Am J Cardiol*. 2001;87:330-332.
12. Ostrowski R, Travis J, Talley E. The association of Hp1 and sickle cell disease. *Hum Hered*. 1987;37:193-195.
13. Moreira H, Naoum P. Serum haptoglobin types in patients with hemoglobinopathies. *Hereditas*. 1990;113:227-231.
14. Hughes H, Kutlar F, Clair B, Elam D, McKie KM, Kutlar A. Haptoglobin genotypes among pediatric and adult sickle cell patients: an emerging genetic modifier? *Blood*. 2005;106(11):3805.
15. Chintagari N, Nguyen J, Belcher J, Vercellotti G, Alayash A. Haptoglobin attenuates hemoglobin-induced heme oxygenase-1 in renal proximal tubule cells and kidneys of a mouse model of sickle cell disease. *Blood Cells Mol Dis*. 2015;54:302-306.
16. Hamdy G, Olfat MH, Hala M, Azza E-S, Salwa RA, Fatma AK. Haptoglobin phenotypes as a risk factor for coronary artery disease in type 2 diabetes mellitus: an Egyptian study. *Egypt J Med Hum Genet*. 2014;15:257-264.
17. Grundy SM, Pasternak R, Greenland P, Smith S, Fuster V. Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations. *J Am Coll Cardiol*. 1999;34:1348-1359.



18. Benzie I, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal Biochem.* 1996; 239:70-76.
19. Sinha A. Colorimetric assay of catalase. *Anal Biochem.* 1972;47(2): 389-394.
20. Folch J, Lees M, Stanley G. A simple method for the isolation and purification and total lipid from animal tissues. *J Biol Chem.* 1957;226:497-509.
21. Ellman GL. Tissue sulphhydryl groups. *Arch Biochem Biophys.* 1959;82: 70-77.
22. Plowe CV, Djimde A, Bouare M, Doumbo O, Wellems TE. Pyrimethamine and proguanil resistance-conferring mutations in plasmodium falciparum dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa. *Am J Trop Med Hyg.* 1995;52(6): 565-568.
23. Houwing ME, De Pagter PJ, Van Beers EJ, et al. Sickle cell disease: clinical presentation and management of a global health challenge. *Blood Rev.* 2019;37:1-52.
24. Dahmani F, Benkirane S, Kouzih J, Woumki A, Mamad H, Masrar A. Etude de l'hémogramme dans la drépanocytose homozygote: à propos de 87 patients. *Pan Afr Med J.* 2016;25:240-250.
25. Kaur M, Dangi CBS, Singh M. An overview on sickle cell disease profile. *Asian J Pharm Clin Res.* 2013;6(1):25-37.
26. Prakash H, Adinath S, Aarti K, Rahul G, Maya V. Antioxidant status and lipid peroxidation in sickle cell anaemia. *Biomed Res.* 2010;21(4): 461-464.
27. Ama Moor V, Pieme C, Chetcha B, et al. Oxidative profile of sickle cell patients in a Cameroonian urban hospital. *BMC Clin Pathol.* 2016;16(15):1-5.
28. Eman R, Amina A-S, Hanan F, Walaa S, Marwa M, Sherien M. Oxidative stress and cytokine role of patients with sickle cell disease. *J Chem Pharm Res.* 2016;8(1):434-438.
29. Amer J, Ghoti H, Rachmilewitz E, Koren A, Levin C, Fibach E. Red blood cells, platelets and polymorphonuclear neutrophils of patients with sickle cell disease exhibit oxidative stress that can be ameliorated by antioxidants. *Br J Haematol.* 2006;132:108-113.
30. Dasgupta T, Hebbel P, Kaul K. Protective effect of arginine on oxidative stress in transgenic sickle mouse models. *Free Radic Biol Med.* 2006;41(12):1771-1780.
31. Alsultan AI, Seif MA, Amin TT, Naboli M, Alsuliman AM. Relationship between oxidative stress, ferritin and insulin resistance in sickle cell disease. *Eur Rev Med Pharmacol Sci.* 2010;14:527-538.
32. Rosa V, Sposi NM, Mattia L, Gambardella L, Straface E, Pietraforte D. Sickle cell disease: role of oxidative stress and antioxidant therapy. *Antioxidants.* 2021;10(296):1-27.
33. Jain SK, Ross JD, Duett J, Herbst JJ. Low plasma prealbumin and carotenoid levels in sickle cell disease patients. *Am J Med Sci.* 1990;299(1):13-15.
34. Kengne FC, Biapa NP, Chetcha B, Nguelwou LF, Pieme CA, Gatsing D. Electrolytic and oxidative stress profile of sickle cell anaemia patients in Cameroon: the effect of some extrinsic factors. *Asian Hematol Res J.* 2018;1(1):1-11.
35. Faes C, Balayssac-Siransy E, Connes P, et al. Moderate endurance exercise in patients with sickle cell anaemia: effects on oxidative stress and endothelial activation. *Br J Haematol.* 2014;164:124-130.
36. Voskou S, Aslan M, Fanis P, Phylactides M, Kleanthous M. Oxidative stress in  $\beta$ -thalassaemia and sickle cell disease. *Redox Biol.* 2015;6:226-239.
37. Mokondjimobe E, Guie G, Bongo N, et al. Profil des lipides plasmatiques chez les drepanocytaires homozygotes et heterozygotes congolais. *Annales de l'Université Marien NGOUABI.* 2010;11(5):37-41.
38. Mondé AA, Kouamé-Koutouan A, Tiahou GG, et al. Profil lipidoprotéinique, isotopique et risque athérogène dans la drépanocytose en Côte d'Ivoire. *Méd Nucl.* 2010;34S:e17-e21.
39. Hama AH, Shakiba E, Rahimi Z, Karimi M, Mozafari H, Abdulkarim OA. Vitamin D level, lipid profile, and vitamin D receptor and transporter gene variants in sickle cell disease patients from Kurdistan of Iraq. *J Clin Lab Anal.* 2021;35:1-7.
40. Kato GJ, Martyr S, Blackwelder WC, et al. Levels of soluble endothelium-derived adhesion molecules in patients with sickle cell disease are associated with pulmonary hypertension, organ dysfunction, and mortality. *Br J Haematol.* 2005;130:943-953.
41. Buchowski MS, Swift LL, Akohoue SA, Shankar SM, Flakoll PJ, Abumrad N. Defects in postabsorptive plasma homeostasis of fatty acids in sickle cell disease. *J Parenter Enteral Nutr.* 2007;31:263-268.
42. Shores J, Peterson J, VanderJagt D, Glew RH. Reduced cholesterol levels in African-American adults with sickle cell disease. *J Natl Med Assoc.* 2003;95:813-817.
43. Olatunya OS, Dulcinea MA, Magnun NN, Tolorunju SK, Adekunle A, Fernando FC. Haptoglobin gene polymorphism in patients with sickle cell anemia: findings from a Nigerian cohort study. *Appl Clin Genet.* 2020;13:107-114.
44. Khalid HO, Khalil HB. Haptoglobin phenotypes distribution among sickle cell anaemia patients with different genotypes. *Eur Acad Res.* 2015;3(6):6587-6598.
45. Bruna S, Perla V, Sandra SM, Samuel AA, Grazielle M, Maria SF. Haptoglobin gene polymorphisms and interleukin-6 and -8 levels in patients with sickle cell anaemia. *Braz J Hematol Hemother.* 2015; 37(5):329-335.
46. Atkinson SH, Mwangi WM, Uyoga SM, et al. The Haptoglobin 2-2 genotype is associated with a reduced incidence of *plasmodium falciparum* malaria in children on the coast of Kenya. *Clin Infect Dis.* 2007;44:802-809.
47. Quaye IK, Ekuban FA, Goka BQ, et al. Haptoglobin 1-1 is associated with susceptibility to severe *plasmodium falciparum* malaria. *Trans R Soc Trop Med Hyg.* 2000;94:216-219.
48. Elagib AA, Kider AO, Akerström B, Elbashir MI. Association of haptoglobin phenotype (1-1) with falciparum malaria in Sudan. *Trans R Soc Trop Med Hyg.* 1998;92:309-311.
49. Atkinson SH, Rockett K, Sirugo G. Seasonal childhood anaemia in West Africa is associated with the haptoglobin 2-2 genotype. *PLoS Med.* 2006;3:172.
50. Cox SE, Makani J, Soka D, et al. Haptoglobin, alpha-thalassaemia and glucose-6-phosphate dehydrogenase polymorphisms and risk of abnormal transcranial doppler among patients with sickle cell anaemia in Tanzania. *Br J Haematol.* 2014;165:699-706.
51. Kasvosve I, Speeckaert MM, Speeckaert R, Masukume G, Delanghe J. Haptoglobin polymorphism and infection. *Adv Clin Chem.* 2010;50: 23-46.
52. Asleh R, Levy AP. In vivo and in vitro studies establishing haptoglobin as a major susceptibility gene for diabetic vascular disease. *Vasc Health Risk Manag.* 2005;1(1):19-28.
53. Levy A, Hochberg I, Jablonski K, et al. Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: the strong heart study. *J Am Coll Cardiol.* 2002;40(11):1984-1990.
54. Sadrzadeh H, Bozorgmehr J. Haptoglobin phenotypes in health and disorders. *Am J Clin Pathol.* 2004;121(1):97-104.
55. Merkler A, Sertić J, Bazina Martinović A, et al. Haptoglobin genotype 2-2 associated with atherosclerosis in patients with ischemic stroke. *Gene.* 2020;752:1-6.
56. Aghaalkhani N, Zamani M, Allameh A, et al. Involvement of haptoglobin phenotypes and genotypes in non-muscle invasive bladder cancer: a possible prognostic marker for risk stratification. *Excli J.* 2020;752:351-359.
57. Ricotti R, De Feudis M, Peri C, et al. Haptoglobin phenotypes are associated with the postload glucose and insulin levels in pediatric obesity. *Int J Endocrinol.* 2020;20:1-8.

**How to cite this article:** Kengne Fotsing CB, Pieme CA, Biapa Nya PC, et al. Relation between haptoglobin polymorphism and oxidative stress status, lipid profile, and cardiovascular risk in sickle cell anemia patients. *Health Sci Rep.* 2022;5:e465. doi:10.1002/hsr.2465