Scientific Research Publishing

Advances in Biological Chemistry, 2023, 13, 171-181
https://www.scirp.org/journal/abc
ISSN Online: 2162-2191
ISSN Print: 2162-2183

Influence of Haptoglobin and Hemoglobin Phenotypic Polymorphisms on Sickle Cell Disease Morbidity

Hugues Ahiboh[1*], Akissi Joelle Koffi[1], Aniéla Kanga[2], Philemond By[1], Fatoumata Koné[3], Hermance Kassi[4], Francisk Kouakou[1], Marie-Laure Hauhouot-Attoungbré[5], Duni Sawadogo[6]

[1]Biochemistry and Clinical Chemistry Unit, CeDReS, University Hospital of Treichville, Abidjan, Ivory Coast
[2]Department of Hematology, University Hospital of Bouaké, Bouaké, Ivory Coast
[3]Molecular Biology Unit, CeDReS, University Hospital of Treichville, Abidjan, Ivory Coast
[4]Hematology Unit, CeDReS, University Hospital of Treichville, Abidjan, Ivory Coast
[5]Department of Biochemistry, Clinical Chemistry and Molecular Biology, Faculty of Pharmaceutical and Biological Sciences, University Felix Houphouet-Boigny, Abidjan, Ivory Coast
[6]Department of Hematology and Cell Biology, Faculty of Pharmaceutical and Biological Sciences, University Felix Houphouet-Boigny, Abidjan, Ivory Coast
Email: *hugues.ahiboh@cedres.org

How to cite this paper: Ahiboh, H., Koffi, A.J., Kanga, A., By, P., Koné, F., Kassi, H., Kouakou, F., Hauhouot-Attoungbré, M.-L. and Sawadogo, D. (2023) Influence of Haptoglobin and Hemoglobin Phenotypic Polymorphisms on Sickle Cell Disease Morbidity. *Advances in Biological Chemistry*, **13**, 171-181.
https://doi.org/10.4236/abc.2023.135012

Received: June 30, 2023
Accepted: October 8, 2023
Published: October 11, 2023

Abstract

Objectives: Sickle cell disease (SCD) has a varied clinical and biological expression depending on the hemoglobin phenotype: $SSFA_2$, SFA_2, $SAFA_2$ and SC. Considering the antioxidant properties of the different haptoglobin phenotypes (Hp 1-1, Hp 2-1, Hp 2-2), it seemed relevant to know their influence on the morbidity of the different hemoglobin phenotype of SCD. Thus, the objective of this study was to identify associations between haptoglobin phenotype and morbidity of different SCD phenotypes. **Methods:** In a retrospective cross-sectional descriptive and analytical study, with a cohort of 170 black African carriers of hemoglobin S, in Ivory Coast, West Africa, hemoglobin and haptoglobin phenotypes were determined by electrophoretic methods. **Results:** The three major phenotypes of haptoglobin polymorphism were found in the SCD cohort: Hp 1-1 (24.1%), Hp 2-1 (56.5%), Hp 2-2 (19.4%). Vaso-occlusions were associated with haptoglobin phenotype Hp 1-1, (OR = 2.03; $CI_{95\%}$ = [1.06 - 3.9]; $p < 0.05$). Probability of worse morbidity score was 4.55 times greater for hemoglobin phenotype different from $SSFA_2$ ($CI_{95\%}$ = [1.43 - 14.44]) and the probability of having the Hp 1-1 phenotype was lower ($CI_{95\%}$ = [0.170 - 0.705]). **Conclusions**: Haptoglobin phenotype was associated to morbidity-adjusted hemoglobin phenotype. The study revealed a greater probability of a worse morbidity when the hemoglobin phenotype is homozygous. Unexpectedly, the worse morbidity is associated to

Hp 1-1 haptoglobin phenotype, the most powerful antioxidant within the different haptoglobin phenotypes. Associations found were not systematic and need further studies to enlighten the determinism of SCD morbidity.

Keywords

Haptoglobin Phenotype, Hemoglobin Phenotype, Sickle Cell Disease, Morbidity

1. Introduction

Sickle cell disease (SCD) is the most widespread genetic disease in the world: the prevalence of the S gene varies within 2% and 30% depending on the population [1]. It is associated with high morbidity and mortality. In Côte d'Ivoire, 12% of the population carries hemoglobin (Hb) S, making this disease a public health problem [2].

The morbid events of the pathology are due to polymerization of globular Hb which induces vascular occlusions and chronic hemolysis. This hemolysis exposes all tissues to the deleterious oxidative effects of hemoglobin [3]. The most common hemoglobin phenotypes that experience Hb S polymerization are $SSFA_2$, SFA_2, $SAFA_2$ and SC. SCD has a varied clinical and biological expression depending on the patient and the hemoglobin phenotype [4].

Haptoglobin (Hp) is a protein with a potent antioxidant activity. The intensity of the antioxidant activity varies according to the haptoglobin phenotypes (Hp 1-1, Hp 2-1, Hp 2-2) [5]. Since haptoglobin binds to extra-globular hemoglobin to attenuate hemoglobin deleterious oxidative stress on tissues, the morbidity of the major forms of sickle cell disease could depend on the phenotype of haptoglobin [6] [7]. Consequently, persons with some haptoglobin phenotypes seem to be more sensitive to some diseases and/or they could have specific prognosis [8] [9] [10]. To date, the determinants of the associations between the haptoglobin phenotype and the clinical and biological manifestations of sickle cell disease are unclear [11] [12]. Therefore, it would be relevant to know the influence of haptoglobin phenotypes on the morbidity of the different profiles of SCD.

The objective of this study was to identify associations between the haptoglobin phenotype and the morbidity of sickle cell diseases. In a specific way, we described the distributions of the clinical and biological profiles of sickle cell disease patients according to their respective hemoglobin and haptoglobin phenotypes.

2. Material and Methods

2.1. Study Design

Using a retrospective cross-sectional descriptive and analytical study, we determined the likely associations between the haptoglobin phenotype and morbidi-

ty-adjusted hemoglobin phenotype in a SCD population.

2.2. Population, Variables and Definitions

The studied population was a cohort of 170 black African patients, carriers of hemoglobin S. The cohort was built in 2021. Patients were taken care in the hematology department of Yopougon University Hospital (Abidjan, Ivory Coast). The biological analyzes were carried out in the biology laboratory of the Yopougon University Hospital and at the Center for Diagnosis and Research on Aids and other infectious diseases (CeDReS), University Hospital of Treichville.

Selection criteria and morbidity were determined with different types and sub-types of collected variables: social, anthropological, clinical and biological variables (Table 1). An overall morbidity score was defined as following. One point was assigned to each clinical, surgical, or infectious complication. Overall morbidity was determined by cumulated morbidities. The morbidity score range was 0 to 10. The higher the morbidity score was, the higher the morbidity was.

Patients regardless their age and their biological sex consulting for medical follow-up of SCD were included in the study. Included hemoglobin phenotypes were homozygous sickle cell disease ($SSFA_2$), sickle-β^+-thalassemia ($SAFA_2$),

Table 1. Variables for selection criteria and morbidity disorders.

Type of variable	Sub-type of variable	Variables	Type of variable value	Sources	Timestamp of data collection
Social and anthropological	Anthropological variables	age, weight, height	scale values	Medical files	Prior to inclusion in cohort
		Biological sex	binary value		
	Social variables	Education level, professional occupation, smoking, alcohol consumption	binary values		
Medical	Non-infectious variables	vaso-occlusions, infectious syndrome, acute hemolytic anemia, acute abdominal syndrome, acute chest syndrome, priapism, stroke, myocardial infarction, diabetes mellitus, arterial hypertension, renal failure, vaccination status	binary values	Medical files	Prior to inclusion in cohort
	Infectious complications	urinary tract infections, bone and joint infections, meningitis, sepsis, ENT infections, severe malaria	binary values	Medical files	
Surgery	Surgical variables	splenectomy, cholecystectomy, osteonecrosis, cholelithiasis	binary values	Medical files	Prior to inclusion in cohort
Biology	Blood assessment of organs and metabolism	urea, creatinine, transaminases, CRP, amylase, blood count, prothrombin level, cephalin level with activator, d-dimers, *etc.*…	scale values	Laboratory analyses	At inclusion in the cohort
	Genetics	Haptoglobin phenotype Hemoglobin phenotype	nominal values	Laboratory analyses	At inclusion in the cohort
Morbidity		Morbidity score	scale value		At inclusion in the cohort

sickle-β^0 thalassemia (SFA_2) and SC hemoglobinosis. Data were collected from patients' medical files. Were excluded from the study, heterozygous Hb AS patients, patients from which we did not get their consent and those who demanded to quit the study.

2.3. Ethical Approval

The study was designed and conducted following the Declaration of Helsinki. It was reviewed and approved by the scientific committee of the medical biology chair of Pharmaceutical and Biological Sciences faculty (University Felix Houphouet-Boigny) and by the medical committee of the Yopougon University Hospital.

3. Analytical Methods

3.1. Phenotyping

At inclusion in the cohort, blood samples with anticoagulant EDTA were collected from fasting patients for at least 10 hours. Hemoglobin phenotyping was performed on whole blood, that of haptoglobin on plasma.

Phenotypes were determined by electrophoretic methods. Hemoglobin electrophoresis was performed on agarose gels at alkaline and acid pH [13]. Haptoglobin electrophoresis was performed on a non-denaturing 5% polyacrylamide vertical gel. Migrations were revealed by the peroxidase activity of the haptoglobin-hemoglobin complex [14].

3.2. Statistics

Probabilities of events were determined, and margins of error were calculated using statistical tests of the IBM SPSS™ v18.0.0 software. Descriptive analyzes described the profile of the studied population. The statistical parameters of the associations between the haptoglobin phenotype and the elements of morbidity or the different phenotypes of SCD were the Pearson's *chi-square* test, the *odd ratio* determined from binary logistic regressions and contingency tables on which were applied the Cochran-Mantel-Haenszel decision test.

A result was considered statistically significant for a p-value < 0.05.

4. Results

4.1. Social and Anthropological Description of the Population

In the cohort, 63.5% were female. Patients' age ranged from 1 to 67 years. The mean age was 18 and the median was 14. The age distribution was skewed to the right (skewness of 1.30). In the population, 85% was literate.

4.2. Clinical and Biological Description of the Population

Clinical disorders of SCD were heterogeneous but the more frequent ones were non-vaso-occlusion hematological disorders (42.4%), non-malarial infectious syndromes (42.3%), vaso-occlusions (28.8%) and severe malaria (18.8%).

4.3. Hemoglobin Phenotypes of the Population

The distribution of different hemoglobin phenotypes in the sickle cell population was as follows: homozygous sickle cell disease $SSFA_2$ (36.5%), hemoglobinosis SC (26.5%), sickle cell-β^+ thalassemia $SAFA_2$ (14.1%) and sickle cell-β^0 thalassemia $SAFA_2$ (22.9%).

4.4. Distribution of Haptoglobin Phenotypes

The major phenotypes of haptoglobin polymorphism were found in our SCD cohort. Distribution of haptoglobin phenotypes in sickle cell disease population was as follows: Hp 1-1 (24.1%), Hp 2-1 (56.5%), Hp 2-2 (19.4%). The phenotype Hp 0-0 was not found.

4.5. Interdependence of Studied Variables

Using adjusted logistic regressions, relationships were sought between occurrence of vaso-occlusions, infectious syndromes or hemolytic crises (considered as dependent variables) and explanatory variables of the study (haptoglobin phenotype, hemoglobin phenotype, education level, vaccination status, *etc*…).

4.5.1. Factors Influencing Occurrences of Vaso-Occlusions

Vaso-occlusions were statistically associated with haptoglobin phenotype Hp 1-1 (Table 2). The probability of having a vaso-occlusive crisis was 2.5 times greater when the haptoglobin phenotype was Hp 1-1 (Table 2).

In univariate analyses, no relationships were found between the occurrence of vaso-occlusions and respectively homozygous phenotype of sickle cell disease, severe malaria and vaccination status. However, relationships appeared between the occurrence of vaso-occlusions and respectively sickle-β^+ thalassemia phenotype ($SAFA_2$) and the infectious syndromes (Table 3).

4.5.2. Factors Influencing Occurrences of Infectious Syndrome

In univariate analyses, no relationship was found between the occurrence of infectious episodes and the haptoglobin phenotype, neither homozygous sickle cell disease. However, there were inverse relationships between the occurrence of an infectious syndrome and respectively the fact of being literate or the vaccination status (Table 4).

Table 2. Distribution of vaso-occlusions according to haptoglobin phenotype.

			Vaso-occlusions	
			no	yes
Haptoglobine phenotype	Hp 1-1	Effective	28	13
		% within haptoglobin phenotype	68.3%	31.7%
	Hp 2-1	Effective	81	15
		% within haptoglobin phenotype	84.4%	15.6%

Hp: Haptoglobin phenotype. More vaso-occlusions when Hp 1-1; OR = 2.03; $CI_{95\%}$ = [1.06 - 3.9], $p < 0.05$.

Table 3. Relationships between vaso-occlusions and different explanatory variables.

		p-value	Odd Ratio	95% Confidence Interval	
				Inferior	Superior
Explanatory variables	Hp 1-1	***0.015****	2.516	1.099	5.760
	Hb $SSFA_2$	*0.078*	1.835	0.830	4.058
	Hb $SAFA_2$	***0.040****	0.140	0.024	0.917
	Infectious syndrome	***0.000****	14.99	4.604	48.815
	Severe malaria	*0.607*	1.976	0.538	7.265
	Vaccination status (partial or completed)	*0.144*	2.190	0.766	6.261

*: $p < 0.05$ (significant difference). Hp: Haptoglobin phenotype; $SSFA_2$: hemoglobin phenotype of homozygous sickle cell disease; $SAFA_2$: hemoglobin phenotype of sickle-β^+ thalassemia.

Table 4. Relationships between the occurrence of infectious syndromes and different explanatory variables.

		p	Odd Ratio	95% Confidence Interval	
				Inferior	Inferior
Explanatory variables	Hp 1-1	*0.913*	0.861	0.299	2.479
	Hb $SSFA_2$	*0.137*	1.835	0.830	4.058
	Literate	***0.031****	0.140	0.024	0.917
	Vaccination status (partial or completed)	***0.008****	0.246	0.088	0.685

*: $p < 0.05$ (significant difference). Hp: haptoglobin phenotype; $SSFA_2$: homozygous sickle cell hemoglobin phenotype.

4.5.3. Factors Affecting the Occurrence of Acute Hemolytic Crises

In univariate analyses, there were significant relationships between the occurrence of acute hemolytic crises and respectively the hemoglobin phenotype, the occurrence of vaso-occlusive episodes or the occurrence of infectious syndrome (Table 5).

4.5.4. Relationship between Haptoglobin and Hemoglobin Phenotypes

In univariate analyses, no direct relationships appeared between haptoglobin phenotype and hemoglobin phenotype.

Multivariate analyses revealed that haptoglobin phenotype was associated to morbidity-adjusted hemoglobin phenotype. When the hemoglobin phenotype was not $SSFA_2$ (homozygous sickle cell disease), the probability of having a lower morbidity score (≤5) was 4.55 times greater. When the hemoglobin phenotype was not $SSFA_2$ (homozygous sickle cell disease), the probability of having the Hp 1-1 phenotype was lower (Table 6).

Table 5. Relationships between the occurrence of hemolytic crises and different explanatory variables.

		Odd Ratio	p
Explanatory variables	Hp 1-1	3.073	*0.080*
	Hb $SSFA_2$	5.379	***0.020****
	Literate	0.033	*0.857*
	Completed vaccination	2.099	*0.147*
	Vaso-occlusions	17.338	***0.000****
	Infectious syndromes	37.286	***0.000****

*: $p < 0.05$ (significant difference). Hp: Haptoglobin phenotype; Hb $SSFA_2$: hemoglobin phenotype of homozygous sickle cell disease.

Table 6. Distribution of haptoglobin phenotype according to morbidity-adjusted hemoglobin phenotype.

Hb phenotype				Haptoglobin phenotype	
				non Hp 1-1	**Hp 1-1**
$SAFA_2$, SFA_2, SC (Heterozygous sickle cell disease, sickle thalassemia)	**Categorical morbidity**	≤5	Effective	78	15
			% within categorical morbidity	83.9%	16.1%
		>5	Effective	8	7
			% within categorical morbidity	53.3%	46.7%
$SSFA_2$ (Homozygous sickle cell disease)	**Categorical morbidity**	≤5	Effective	31	16
			% within categorical morbidity	66.0%	34.0%
		>5	Effective	12	3
			% within categorical morbidity	80.0%	20.0%

Hp: Haptoglobin phenotype; Hb: hemoglobin; Fisher's exact test for Hb phenotypes other than $SSFA_2$; $p < 0.05$; Odds Ratio for Hb non $SSFA_2$ phenotype for morbidity ≤ 5: 4.55 $CI_{95\%}$ = [1.43 - 14.44]; Odds Ratio for phenotype Hb non $SSFA_2$ for Hp 1-1: 0.346 $CI_{95\%}$ = [0.170 - 0.705].

5. Discussion

5.1. About Analytical Methods

Previous studies have matched the electrophoretic fingerprint of phenotyping with the PCR method of haptoglobin genotyping [15]. Therefore, the electrophoretic phenotyping of haptoglobin makes it possible to highlight the phenotypic polymorphism of the haptoglobin gene.

5.2. About the Studied Population

SCD is a genetic disorder with variable morbidity and mortality depending on the genetic profile and the quality of medical care [16] [17]. This contributes to a right-side skewed age distribution.

According to many studies, the average age of homozygous sickle cell disease (Hb SS) is 25 to 27 years old [18] [19]. However, the average age of our population was 18 years. This difference could be explained on the one hand, by the

phenotypic heterogeneity of Hb in our population ($SSFA_2$, SFA_2, $SAFA_2$, SC) and on the other hand, by possible differences in the quality of medical follow-up.

Morbidity disorders were dominated by hematological disorders and infectious syndromes. It is known that the literacy rate lowers morbidity [20] [21]. However, morbidity in our study, specifically the frequency of the infectious syndrome, is high despite a literacy rate of 85%. Factors other than the literacy rate seem to be associated with this morbidity.

In the studied population, the clinical and biological symptoms of SCD, even though varied, were dominated by a pathophysiology inducing blood transfusions, vaso-occlusions and infectious syndromes like in previous studies [22] [23].

5.3. About Haptoglobin Phenotypes

Phenotyping by the electrophoretic method detects the Hp 0-0 phenotype, but it does not fit with the differentiation of the Hp 0-0 phenotype by acquired hypohaptoglobinemia from the Hp 0-0 phenotype by congenital anhaptoglobinemia. However, the Hp 0-0 phenotype was not found in our population, although described in black African populations [24].

Although one of the roles of haptoglobin is to inhibit extracorpuscular hemoglobin, no direct association between the hemoglobin phenotype and the haptoglobin phenotype was revealed in the studied population. However, in multivariate analyses, hemoglobin phenotype-adjusted morbidity appeared to vary with haptoglobin phenotype in SCD (**Table 6**). When the hemoglobin phenotype was not homozygous, morbidity was lower, with a greater probability for the haptoglobin Hp 2-1 and Hp 2-2 phenotypes. Thereby, the conjunction of a heterozygous SCD (non-$SSFA_2$ phenotype) and a haptoglobin phenotype different from Hp 1-1 (Hp 2-1 or Hp 2-2) appeared to be a better prognostic factor (based on the morbidity score). On the contrary, in Meher's study, whose population was only homozygous sickle cell patients (Hb $SSFA_2$), it was the Hp 2-2 phenotypes that had the worst prognosis [25]. Like our results, Fotsing also showed, in a population of homozygous sickle cell subjects, that subjects with the Hp 1-1 phenotype had a greater tendency to oxidative stress than Hp 2-1 subjects [26]. Since several studies present the Hp^2 allele associated with phenotypes of lower antioxidant activity, our results suggest further research to understand the reason why the Hp^1 allele is associated with greater morbidity in this study.

The association between haptoglobin phenotype and SCD morbidity may involve other factors not considered in the present study. Simultaneous description of the genetic profile, immunoinflammatory status, and haptoglobin phenotype could enlighten the determinants of morbidity in SCD and other similar genetic conditions.

6. Conclusion

The major haptoglobin phenotypes were found in the SCD population. An asso-

ciation between morbidity and the haptoglobin phenotype appeared. In a SCD, there was a greater probability of presenting a worse morbidity when the hemoglobin phenotype is homozygous and when the haptoglobin phenotype is Hp 1-1. However, the associations found were not systematic and need further studies to provide more insight in the determinism of SCD morbidity.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Osunkwo, I., Andemariam, B., Minniti, C.P., Inusa, B.P.D., El Rassi, F., Francis-Gibson, B., *et al.* (2021) Impact of Sickle Cell Disease on Patients' Daily Lives, Symptoms Reported, and Disease Management Strategies: Results from the International Sickle Cell World Assessment Survey (SWAY). *American Journal of Hematology*, **96**, 404-417. https://doi.org/10.1002/ajh.26063

[2] Tolo-Diebkilé, A., Koffi, K.G., Nanho, D.C., Sawadogo, D., Kouakou, B., Siransy-Bogui, L., *et al.* (2010) Homozygous Sickle Cell Disease in Ivorian Adults over 21 Years Old. *Cahiers d'études et de recherches francophones/Santé*, **20**, 63-67. https://doi.org/10.1684/san.2010.0184

[3] Dubert, M., Elion, J., Tolo, A., Diallo, D.A., Diop, S., Diagne, I., *et al.* (2017) Degree of Anemia, Indirect Markers of Hemolysis, and Vascular Complications of Sickle Cell Disease in Africa. *Blood*, **130**, 2215-2223. https://doi.org/10.1182/blood-2016-12-755777

[4] Rees, D.C., Williams, T.N. and Gladwin, M.T. (2010) Sickle-Cell Disease. *The Lancet*, **376**, 2018-2031. https://doi.org/10.1016/S0140-6736(10)61029-X

[5] Moreira, L.R.S., Miranda-Vilela, A.L., Silva, I.C.R., Akimoto, A.K., *et al.* (2009) Antioxidant Effect of Haptoglobin Phenotypes against DNA Damage Induced by Hydrogen Peroxide in Human Leukocytes. *Genetics and Molecular Research*, **8**, 284-290. https://doi.org/10.4238/vol8-1gmr569

[6] Gueye Tall, F., Martin, C., Ndour, E.H.M., Faes, C., Déme Ly, I., Pialoux, V., *et al.* (2020) Influence of Oxidative Stress Biomarkers and Genetic Polymorphisms on the Clinical Severity of Hydroxyurea-Free Senegalese Children with Sickle Cell Anemia. *Antioxidants*, **9**, Article 863. https://doi.org/10.3390/antiox9090863

[7] Guéye, P.M., Glasser, N., Férard, G. and Lessinger, J.M. (2006) Influence of Human Haptoglobin Polymorphism on Oxidative Stress Induced by Free Hemoglobin on Red Blood Cells. *Clinical Chemistry and Laboratory Medicine*, **44**, 542-547. https://doi.org/10.1515/CCLM.2006.095

[8] Naryzny, S.N. and Legina, O.K. (2021) Haptoglobin as a Biomarker. *Biochemistry* (*Moscow*), *Supplement Series B*: *Biomedical Chemistry*, **15**, 184-198. https://doi.org/10.1134/S1990750821030069

[9] Madkour, M.I., Hassan, R.E., Sherif, N.M., Awadallah, S., Abdelrahim, D.N., Jahrami, H.A., *et al.* (2022) Haptoglobin Polymorphism Modulates Cardiometabolic Impacts of Four Consecutive Weeks, Dawn to Sunset Ramadan Intermittent Fasting among Subjects with Overweight/Obesity. *Diabetes Research and Clinical Practice*, **190**, Article ID: 110024. https://doi.org/10.1016/j.diabres.2022.110024

https://www.diabetesresearchclinicalpractice.com/article/S0168-8227(22)00838-5/fulltext

[10] Edwards, O., Burris, A., Lua, J., Wilkie, D.J., Ezenwa, M.O. and Doré, S. (2022) Influence of Haptoglobin Polymorphism on Stroke in Sickle Cell Disease Patients. *Genes*, **13**, Article 144. https://doi.org/10.3390/genes13010144

[11] Marshall, K., Howell, S., Badaloo, A., Reid, M., McFarlane-Anderson, N. and McKenzie, C. (2018) Exploring Putative Genetic Determinants of Inter-Individual Phenotypic Heterogeneity in Sickle Cell Disease: A Cross-Sectional Jamaican Cohort-Based Study. *Blood Cells, Molecules, and Diseases*, **73**, 1-8. https://doi.org/10.1016/j.bcmd.2018.08.001

[12] Adekile, A.D. and Haider, M.Z. (2010) Haptoglobin Gene Polymorphisms in Sickle Cell Disease Patients with Different β^S-Globin Gene Haplotypes. *Medical Principles and Practice*, **19**, 447-450. https://doi.org/10.1159/000320302

[13] Louderback, A.L. and Shanbrom, E. (1967) Hemoglobin Electrophoresis. *The Journal of the American Medical Association*, **202**, 718-719. https://doi.org/10.1001/jama.202.8.718

[14] Engler, R., Rondeau, Y., Pointis, J. and Jayle, M.F. (1973) Peroxydasic Activities of Hemoglobinic Combinations of the Three Haptoglobin Phenotypes. *Clinica Chimica Acta*, **47**, 149-152. https://doi.org/10.1016/0009-8981(73)90309-4

[15] Koch, W., Latz, W., Eichinger, M., Roguin, A., Levy, A.P., Schömig, A., *et al.* (2002) Genotyping of the Common Haptoglobin Hp 1/2 Polymorphism Based on PCR. *Clinical Chemistry*, **48**, 1377-1382. https://doi.org/10.1093/clinchem/48.9.1377

[16] Marks, L.J., Munube, D., Kasirye, P., Mupere, E., Jin, Z., LaRussa, P., *et al.* (2018) Stroke Prevalence in Children with Sickle Cell Disease in Sub-Saharan Africa: A Systematic Review and Meta-Analysis. *Global Pediatric Health*, **5**, 1-9. https://doi.org/10.1177/2333794X18774970

[17] Kato, G.J., Piel, F.B., Reid, C.D., Gaston, M.H., Ohene-Frempong, K., Krishnamurti, L., *et al.* (2018) Sickle Cell Disease. *Nature Reviews Disease Primers*, **4**, Article No. 18010. https://doi.org/10.1038/nrdp.2018.10

[18] Desai, R.J., Mahesri, M., Globe, D., Mutebi, A., Bohn, R., Achebe, M., *et al.* (2020) Clinical Outcomes and Healthcare Utilization in Patients with Sickle Cell Disease: A Nationwide Cohort Study of Medicaid Beneficiaries. *Annals of Hematology*, **99**, 2497-2505. https://doi.org/10.1007/s00277-020-04233-w

[19] Diop, S., Mokono, S.O., Ndiaye, M., Touré Fall, A.O., Thiam, D. and Diakhaté, L. (2003) Homozygous Sickle Cell Disease in Patients above 20 Years of Age: Follow-up of 108 Patients in Dakar. *La Revue de Médecine Interne*, **24**, 711-715. https://doi.org/10.1016/S0248-8663(03)00220-0

[20] Perry Caldwell, E. and Killingsworth, E. (2021) The Health Literacy Disparity in Adolescents with Sickle Cell Disease. *Journal for Specialists in Pediatric Nursing*, **26**, e12353. https://onlinelibrary.wiley.com/doi/10.1111/jspn.12353 https://doi.org/10.1111/jspn.12353

[21] Daak, A.A., Elsamani, E., Ali, E.H., Mohamed, F.A., Abdel-Rahman, M.E., Elderdery, A.Y., *et al.* (2016) Sickle Cell Disease in Western Sudan: Genetic Epidemiology and Predictors of Knowledge Attitude and Practices. *Tropical Medicine & International Health*, **21**, 642-653. https://doi.org/10.1111/tmi.12689

[22] Baltierra, D., Harper, T., Jones, M.P. and Nau, K.C. (2015) Hematologic Disorders: Sickle Cell Disease. *FP Essentials*, **433**, 27-39.

[23] Keber, B., Lam, L., Mumford, J. and Flanagan, B. (2019) Hematologic Conditions: Common Hemoglobinopathies. *FP Essentials*, **485**, 24-31.

[24] Ko, D.H., Chang, H.E., Kim, T.S., Song, E.Y., Park, K.U., Song, J., *et al.* (2013) A Review of Haptoglobin Typing Methods for Disease Association Study and Preventing Anaphylactic Transfusion Reaction. *BioMed Research International*, **2013**, Article ID: 390630. https://doi.org/10.1155/2013/390630

[25] Meher, S., Mohanty, P.K., Patel, S., Das, K., Sahoo, S., Dehury, S., *et al.* (2021) Haptoglobin Genotypes Associated with Vaso-Occlusive Crisis in Sickle Cell Anemia Patients of Eastern India. *Hemoglobin*, **45**, 358-364. https://doi.org/10.1080/03630269.2020.1801459

[26] Kengne Fotsing, C.B., Pieme, C.A., Biapa Nya, P.C., Chedjou, J.P., Dabou, S., Nguemeni, C., *et al.* (2022) Relation between Haptoglobin Polymorphism and Oxidative Stress Status, Lipid Profile, and Cardiovascular Risk in Sickle Cell Anemia Patients. *Health Science Reports*, **5**, e465. https://doi.org/10.1002/hsr2.465