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

Hugues Ahiboh1*, Akissi Joelle Koffi1, Aniêla Kanga2, Philemond By1, Fatoumata Koné3, Hermance Kassi4, Francisk Kouakou1, Marie-Laure Hauhouot-Attoungbré5, Duni Sawadogo6

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

Abstract

Objectives: Sickle cell disease (SCD) has a varied clinical and biological expression depending on the hemoglobin phenotype: SSFA2, SFA2, SAFa2 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; CI95% = [1.06 - 3.9]; p < 0.05). Probability of worse morbidity score was 4.55 times greater for hemoglobin phenotype different from SSFA2 (CI95% = [1.43 - 14.44]) and the probability of having the Hp 1-1 phenotype was lower (CI95% = [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


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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 SSFA2, SFA2, SAFA2 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 analyses 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 (SSFA2), sickle-β⁺-thalassemia (SAFA₂),

### Table 1. Variables for selection criteria and morbidity disorders.

<table>
<thead>
<tr>
<th>Type of variable</th>
<th>Sub-type of variable</th>
<th>Variables</th>
<th>Type of variable value</th>
<th>Sources</th>
<th>Timestamp of data collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social and anthropological variables</td>
<td>Anthropological variables</td>
<td>age, weight, height, Biological sex, Education level, professional occupation, smoking, alcohol consumption</td>
<td>scale values</td>
<td>Medical files</td>
<td>Prior to inclusion in cohort</td>
</tr>
<tr>
<td>Social variables</td>
<td>Medical</td>
<td>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</td>
<td>binary values</td>
<td>Medical files</td>
<td>Prior to inclusion in cohort</td>
</tr>
<tr>
<td>Non-infectious variables</td>
<td>Infectious complications</td>
<td>urinary tract infections, bone and joint infections, meningitis, sepsis, ENT infections, severe malaria</td>
<td>binary values</td>
<td>Medical files</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>Surgical variables</td>
<td>splenectomy, cholecystectomy, osteonecrosis, cholelithiasis</td>
<td>binary values</td>
<td>Medical files</td>
<td>Prior to inclusion in cohort</td>
</tr>
<tr>
<td>Biology</td>
<td>Blood assessment of organs and metabolism</td>
<td>urea, creatinine, transaminases, CRP, amylase, blood count, prothrombin level, cephalin level with activator, d-dimers, etc…</td>
<td>scale values</td>
<td>Laboratory analyses</td>
<td>At inclusion in the cohort</td>
</tr>
<tr>
<td>Genetics</td>
<td>Haptoglobin phenotype, Hemoglobin phenotype</td>
<td></td>
<td>nominal values</td>
<td>Laboratory analyses</td>
<td>At inclusion in the cohort</td>
</tr>
<tr>
<td>Morbidity</td>
<td>Morbidity score</td>
<td>scale value</td>
<td></td>
<td></td>
<td>At inclusion in the cohort</td>
</tr>
</tbody>
</table>
sickle-β\(^{-}\) 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\textsuperscript{TM} 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 SSFA₂ (36.5%), hemoglobinosis SC (26.5%), sickle cell-β⁺ thalassemia SAFA₂ (14.1%) and sickle cell-β⁻ thalassemia SAFA₂ (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 (SAFA₂) 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.

<table>
<thead>
<tr>
<th>Haptoglobin phenotype</th>
<th>Vaso-occlusions</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Hp 1-1</td>
<td>Effective</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>% within haptoglobin phenotype</td>
<td>68.3%</td>
<td>31.7%</td>
</tr>
<tr>
<td>Hp 2-1</td>
<td>Effective</td>
<td>81</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>% within haptoglobin phenotype</td>
<td>84.4%</td>
<td>15.6%</td>
</tr>
</tbody>
</table>

Hp: Haptoglobin phenotype. More vaso-occlusions when Hp 1-1; OR = 2.03; CI95% = [1.06 - 3.9], p < 0.05.
Table 3. Relationships between vaso-occlusions and different explanatory variables.

<table>
<thead>
<tr>
<th>Explanatory variables</th>
<th>p-value</th>
<th>Odd Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inferior</td>
<td>Superior</td>
</tr>
<tr>
<td>Hp 1-1</td>
<td>0.015*</td>
<td>2.516</td>
<td>1.099 5.760</td>
</tr>
<tr>
<td>Hb SSFA₂</td>
<td>0.078</td>
<td>1.835</td>
<td>0.830 4.058</td>
</tr>
<tr>
<td>Hb SAFA₂</td>
<td>0.040*</td>
<td>0.140</td>
<td>0.024 0.917</td>
</tr>
<tr>
<td>Infectious syndrome</td>
<td>0.000*</td>
<td>14.99</td>
<td>4.604 48.815</td>
</tr>
<tr>
<td>Severe malaria</td>
<td>0.607</td>
<td>1.976</td>
<td>0.538 7.265</td>
</tr>
<tr>
<td>Vaccination status</td>
<td>0.144</td>
<td>2.190</td>
<td>0.766 6.261</td>
</tr>
</tbody>
</table>

*: p < 0.05 (significant difference). Hp: Haptoglobin phenotype; SSFA₂: hemoglobin phenotype of homozygous sickle cell disease; SAFA₂: hemoglobin phenotype of sickle-β⁺ thalassemia.

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

<table>
<thead>
<tr>
<th>Explanatory variables</th>
<th>p</th>
<th>Odd Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inferior</td>
<td>Inferior</td>
</tr>
<tr>
<td>Hp 1-1</td>
<td>0.913</td>
<td>0.861</td>
<td>0.299 2.479</td>
</tr>
<tr>
<td>Hb SSFA₂</td>
<td>0.137</td>
<td>1.835</td>
<td>0.830 4.058</td>
</tr>
<tr>
<td>Literate</td>
<td>0.031*</td>
<td>0.140</td>
<td>0.024 0.917</td>
</tr>
<tr>
<td>Vaccination status</td>
<td>0.008*</td>
<td>0.246</td>
<td>0.088 0.685</td>
</tr>
</tbody>
</table>

*: p < 0.05 (significant difference). Hp: haptoglobin phenotype; SSFA₂: 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 SSFA₂ (homozygous sickle cell disease), the probability of having a lower morbidity score (≤5) was 4.55 times greater. When the hemoglobin phenotype was not SSFA₂ (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.

<table>
<thead>
<tr>
<th>Explanatory variables</th>
<th>Odd Ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hp 1-1</td>
<td>3.073</td>
<td>0.080</td>
</tr>
<tr>
<td>Hb SSFA2</td>
<td>5.379</td>
<td>0.020*</td>
</tr>
<tr>
<td>Literate</td>
<td>0.033</td>
<td>0.857</td>
</tr>
<tr>
<td>Completed vaccination</td>
<td>2.099</td>
<td>0.147</td>
</tr>
<tr>
<td>Vaso-occlusions</td>
<td>17.338</td>
<td>0.000*</td>
</tr>
<tr>
<td>Infectious syndromes</td>
<td>37.286</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

*: p < 0.05 (significant difference). Hp: Haptoglobin phenotype; Hb SSFA2: hemoglobin phenotype of homozygous sickle cell disease.

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

<table>
<thead>
<tr>
<th>Hb phenotype</th>
<th>Haptoglobin phenotype</th>
<th>Categorical morbidity</th>
<th>Effective</th>
<th>% within categorical morbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSFA2</td>
<td>non Hp 1-1</td>
<td>≤5</td>
<td>78</td>
<td>83.9%</td>
</tr>
<tr>
<td>SC</td>
<td></td>
<td>≥5</td>
<td>16</td>
<td>16.1%</td>
</tr>
<tr>
<td>SFA2</td>
<td></td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFA2</td>
<td>Hp 1-1</td>
<td>≤5</td>
<td>31</td>
<td>66.0%</td>
</tr>
<tr>
<td>SC</td>
<td></td>
<td>≥5</td>
<td>12</td>
<td>34.0%</td>
</tr>
<tr>
<td>SFA2</td>
<td></td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hp: Haptoglobin phenotype; Hb: hemoglobin; Fisher’s exact test for Hb phenotypes other than SSFA2; p < 0.05; Odds Ratio for Hb non SSFA2 phenotype for morbidity ≤ 5: 4.55 CI95% = [1.43 - 14.44]; Odds Ratio for phenotype Hb non SSFA2 for Hp 1-1: 0.346 CI95% = [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 (SSFA₂, SFA₃, SAFA₂, 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-SSFA₂ 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 SSFA₂), 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 Hp₂ allele associated with phenotypes of lower antioxidant activity, our results suggest further research to understand the reason why the Hp₁ 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


