BIOLOGICAL AND CLINICAL PRESENTATION OF
PATIENTS WITH HEMOGLOBINOPATHIES
ATTENDING AN URBAN HOSPITAL IN
OUAGADOUGOU: CONFIRMATION OF THE
MODIFICATION OF THE BALANCE BETWEEN
Hb S AND Hb C IN BURKINA FASO

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ABSTRACT

The incidence of hemoglobinopathies (Hb C and Hb S) is relatively high in
West Africa. In order to characterize the clinical phenotypes of these
hemoglobinopathies 10,166 subjects were studied for suspected hemoglobin-
opathies at the Laboratory of the Centre Medical Saint Camille (CMSC),
Ouagadougou, Burkina Faso. A high rate of Hb SC (6.49%) and Hb SS
(1.93%) individuals were detected at the CMSC as a consequence of a
selective process, whereby patients with anemia or symptoms of vascular
occlusive crisis underwent blood tests. The higher frequency of Hb SC may be
explained by the fact that this condition is less severe than the SS status, and it
requires frequent clinical and laboratory review. On the other hand, the
frequency of Hb CC is very low because it does not interfere with their health

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status. Moreover, the high percentage of Hb S (12.29%) and Hb C (19.28%) trait individuals may be explained by the fact that, in general, all Hb SS and Hb SC patients followed at the CMSC have parents, siblings and other relatives who could have been referred by the center to receive blood tests. The dramatic increase over the past few years in the prevalence of Hb SS [who were absent in the 1984 study of Labie et al.[5]] and of Hb SC, may be attributed to its reduced lethality due to social and health changes. In conclusion, secondary prevention for the control of concurrent and associated diseases is essential in Hb SS and Hb SC patients for improving health and life expectancy.

INTRODUCTION

Hb SS is characterized by severe anemia and frequent vaso-occlusive manifestations. The mortality is primarily due to infections but the role of Plasmodium (P) falciparum malaria in the younger years of life cannot be ignored.[1] The interaction between $\beta^S$ and $\beta^C$ shows a less severe clinical picture and it is characterized by less severe anemia, if the thrombo-embolic manifestations also remain frequent. From data collected by Livingston in 1991[2] it seems evident that, in the last 30 years, a change in the health status of the different genotypes and therefore their prevalence, has taken place, resulting in a lower percentage of $\beta^C$ and a higher percentage of $\beta^S$.[3] A careful study of the clinical phenotypes of these hemoglobinopathies provides useful information about the effect of recent changes in social and health conditions and precocious diagnosis in Burkina Faso. This is a further confirmation that in this new millennium people affected by sickle cell anemia may have a longer life expectancy and better control of their correlated pathologies in all parts of the world.

MATERIALS AND METHODS

Samples

Ten thousand, one hundred and sixty-six subjects, suspected of having a hemoglobinopathy, living in the urban area of Ouagadougou, Burkina Faso, were investigated in the period of 1997–1999 at the Centre Medical Saint Camille (CMSC) of Ouagadougou, Burkina Faso. Their recruitment is obviously biased and cannot be considered as an epidemiological study, but only an opportunity to observe a large number of homozygotes (Hb SS and Hb CC), compound heterozygotes (Hb SC) and heterozygotes. Some of these subjects were referred to the center for some nonspecific symptoms of disease, others for a familial investigation after discovery of an index case. Sometimes, there was an incidental discovery of an abnormal hemoglobin (Hb) in the course of a health check-up.
before starting a new job. Patients who were Hb SS homozygotes arrived at the CMSC because of a vascular-occlusive crisis and severe anemia; compound heterozygote patients for $\beta^c$ and $\beta^s$ were observed primarily for anemia, but also for vascular occlusive pain.

**Methods**

In all subjects, the screening was performed by Hb electrophoresis using cellulose acetate plates (Helena Laboratories, Beaumont, TX, USA) with pH 8.6 buffer. When abnormal Hbs were detected, citrate agar electrophoresis at pH 6.4 and a solubility test (to confirm the presence of Hb S and Hb C) were performed. The percentage of abnormal Hb was measured by gel densitometry with the use of an ADEL 16 analyzer (Minivolt, Rome, Italy). In 763 subjects, in addition to Hb electrophoresis, a complete evaluation of hematological parameters (RBC, Hb, PCV, MCV, MCH, MCHC, WBC, PLT) and a measure of serum bilirubin was also made.

**RESULTS**

A high incidence of Hb AC (19.28%), Hb AS (12.2%), Hb SC (6.49%), Hb SS (1.93%) and Hb CC (1.88%) was found in the study group. This observation, which must be the consequence of a biased recruitment, corresponds to higher frequencies of $\beta^c$ and $\beta^s$ genes (0.147 and 0.113, respectively), which is higher than the real gene frequencies of $\beta^c$ (0.111) and $\beta^s$ (0.051) in Burkina Faso.\[4\]

This was not an epidemiological study but rather a study looking at phenotypes. The median age of the Hb SS patients was nine years, and the oldest patients observed were 22 years old. The other genotypes had a median age of 11, and the oldest of these was 42 years old. These data may be considered an approximate indication of life expectancy.

All patients found to be homozygous for Hb S at first contact at CMSC showed thrombo-embolic manifestations and some also had severe anemia; all were treated with aspirin and hydrosaline infusion until the symptoms resolved. Others came to the CMSC for pneumonia or for degeneration of the femur head.

Fifty-two point five percent of Hb SS subjects were diagnosed within the first three years of life, 30% between 4 and 15 years, and 17.5% after the age of 16 years. In the Hb SC patients, the diagnosis was made slightly later, and in a few occasions in adulthood, while in the patients with Hb CC, the diagnosis was made primarily when asymptomatic after 15 years of age during a routine health check (Fig. 1). The number of episodes of thrombo-embolism per year in Hb SS was between 2–24, with a median rate of three per year. The first manifestation in 30%
of children involved the metacarpal, metatarsal and proximal phalanges (the so-called hand-foot syndrome). The spleen is markedly enlarged in the first two years, and later it became smaller due to repetitive infarctions.

Seventy percent of Hb SC patients showed rarer and less severe forms of thromboembolism and a less prominent anemia (10.9 ± 0.45 g/dL). On the other hand, they showed splenomegaly during the first five years of life. Infections (pneumonia and osteitis) were frequent in 55% of Hb SS patients and in 21% of Hb SC patients. The patients who were homozygous for Hb CC were in good clinical condition: 8% showed slight cutaneous jaundice, 17% had scleral jaundice and only 15% had splenomegaly of moderate degree.

Table 1 lists the hematological parameters of patients according to genotype. A marked reduction of RBC count was found in AS and especially in SS groups, with less Hb and PCV, while the MCV was reduced in the SC group and the MCHC was higher. Also PLT counts were higher in the SC group, while the WBC count was higher in the CC group. Serum bilirubin (BT) was slightly elevated in Hb SS and Hb CC, but only slightly in Hb SC. In patients with Hb SS hydroxyurea (15–20 mg/Kg/day) and traditional drugs, such as FAGARA and DREPANOSTA, were given occasionally to prevent vascular occlusive crises. Folic acid was prescribed to all subjects, who showed a low level of Hb associated with the presence of Hb S and Hb C. Iron (1 mg/Kg/day) and folic acid (100 mg/day) were given to anemic patients negative for hemoglobinopathies.

Figure 1. Age at diagnosis of hemoglobinopathy in the CMSC.
Table 1. Hematological Parameters of 763 Subjects Studied According to Their Hemoglobin Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>AA ($n = 472$)</th>
<th>AC ($n = 154$)</th>
<th>AS ($n = 80$)</th>
<th>CC ($n = 14$)</th>
<th>SC ($n = 32$)</th>
<th>SS ($n = 11$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC ($10^{12}$/L)</td>
<td>4.34 ± 0.71</td>
<td>4.40 ± 0.80</td>
<td>3.86 ± 1.24$^1$</td>
<td>4.31 ± 0.48</td>
<td>4.27 ± 0.45</td>
<td>3.15 ± 0.80$^1$</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>11.9 ± 2.34</td>
<td>11.6 ± 0.80</td>
<td>10.5 ± 1.24$^1$</td>
<td>11.0 ± 0.48</td>
<td>10.9 ± 0.45$^2$</td>
<td>8.9 ± 2.3$^1$</td>
</tr>
<tr>
<td>PCV (L/L)</td>
<td>0.393 ± 0.07</td>
<td>0.377 ± 0.07</td>
<td>0.341 ± 0.10</td>
<td>0.357 ± 0.05</td>
<td>0.351 ± 0.06</td>
<td>0.250 ± 0.09$^1$</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>91.1 ± 9.19</td>
<td>86.7 ± 9.01</td>
<td>90.1 ± 7.19</td>
<td>83.2 ± 9.82$^2$</td>
<td>81.9 ± 5.79</td>
<td>85.8 ± 8.76</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>27.6 ± 3.80</td>
<td>26.7 ± 3.52</td>
<td>27.2 ± 2.09</td>
<td>25.6 ± 3.49</td>
<td>25.5 ± 1.55</td>
<td>23.6 ± 8.3$^2$</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>30.2 ± 1.80</td>
<td>30.6 ± 1.36</td>
<td>30.5 ± 0.64</td>
<td>30.8 ± 0.69</td>
<td>32.7 ± 1.83$^1$</td>
<td>31.4 ± 1.85$^1$</td>
</tr>
<tr>
<td>WBC ($10^9$/L)</td>
<td>6.31 ± 2.89</td>
<td>7.16 ± 3.64</td>
<td>9.44 ± 8.20$^1$</td>
<td>9.77 ± 4.76$^1$</td>
<td>10.50 ± 4.38$^1$</td>
<td>13.22 ± 4.57$^1$</td>
</tr>
<tr>
<td>PLT ($10^9$/L)</td>
<td>285.0 ± 89.0</td>
<td>300.0 ± 93.0</td>
<td>296.0 ± 93.0</td>
<td>320.0 ± 80.0</td>
<td>345.0 ± 85.0$^1$</td>
<td>262.0 ± 52.6</td>
</tr>
<tr>
<td>TSB (mg%)</td>
<td>0.80 ± 0.45</td>
<td>0.93 ± 0.66</td>
<td>0.85 ± 0.41</td>
<td>1.52 ± 0.79$^1$</td>
<td>1.25 ± 0.63$^1$</td>
<td>1.76 ± 0.67$^1$</td>
</tr>
<tr>
<td>DRB (mg%)</td>
<td>0.40 ± 0.23</td>
<td>0.54 ± 0.22</td>
<td>0.35 ± 0.24</td>
<td>0.76 ± 0.46$^1$</td>
<td>0.75 ± 0.21$^1$</td>
<td>0.80 ± 0.35$^1$</td>
</tr>
<tr>
<td>IRB (mg%)</td>
<td>0.38 ± 0.37</td>
<td>0.46 ± 0.39</td>
<td>0.50 ± 0.36</td>
<td>0.86 ± 0.56$^1$</td>
<td>0.50 ± 0.42</td>
<td>0.95 ± 0.34$^1$</td>
</tr>
</tbody>
</table>

*TSB = total serum bilirubin; DRB = direct reacting bilirubin; IRB = indirect reacting bilirubin.*

$^1$Student $t$-test $p < 0.0001$.

$^2$Student $t$-test $p < 0.001$. 
DISCUSSION

The high number of homozygotes for $\beta^S$ detected in the Ouagadougou population (196/10,166, corresponding to the 1.93% of all subjects studied) as compared to the lack of cases detected by Labie et al. indicates that the improved social and health conditions, the use of vaccines and antibiotic prophylaxis for streptococcus pneumonia infection, have played an important role in establishing a more balanced polymorphism with the Hb S-malaria relationship. Alouch has demonstrated that this relationship is maintained by a higher mortality rate of the Hb AA subjects due to malaria, and the high mortality rate of Hb SS and Hb SC patients caused by complications of their disease, that are better controlled today than in the past.

The higher numbers of Hb SC (660) in this study are a direct consequence of the selection process, whereby patients presenting with anemia or pain due to thrombo-embolic disease underwent blood tests. Moreover, since their life expectancy is higher than that of Hb SS (possibly also through resistance to malaria), Hb SC individuals could contribute to the persistence and expansion of the $\beta^S$ gene in all countries of the Benin Gulf. The high rate of Hb AS (12.29%) and Hb AC (19.28%) individuals in this study may be explained by the fact that all Hb SS and Hb SC patients treated at the CMSC referred their families to the same center for testing. Indeed, the observation that Hb AS individuals are slightly anemic, may be due to the younger age of this group, which was addressed by the CMSC as a consequence of expanded intra-familial screening. Clinically the SS and SC patients show signs of chronic hemolysis and sickle cell disease (both thrombo-embolic phenomenon and pain crisis).

If we consider that patients homozygous for Hb CC are able to live normal lifestyles with no effect on reproductive capacity, then the advantage of $\beta^C$ over $\beta^S$ is improved by the absence of associated pathology. In fact, Hb CC subjects observed at the CMSC, showed hematological parameters within the normal range for the Black population, with the exception of a slightly elevated total bilirubin. Modiano et al. recently demonstrated that Hb C provides protection against clinical $P. falciparum$ malaria in both the heterozygous (29%) and homozygous state (93%), and it represents the most important factor that maintains the $\beta^C$ gene frequency in Burkina Faso.

Life expectancy for patients with Hb SS in Africa can be increased by improving their clinical state. This can be achieved by introduction of new medical strategies such as hydroxyurea, hydration, aimed at reducing the frequency and severity of vascular occlusive crises. The results of this study, which confirms the high rate of abnormal Hbs in Burkina Faso, and their adverse effects on the health status in homozygotes and compound heterozygotes, focus on the need for secondary prevention. The development of sickle cell anemia services, a problem not yet completely solved even in developed countries, becomes particularly important now that patients with sickle cell anemia have a longer life expectancy.
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