

## ORIGINAL ARTICLE (B)

# **Biological and clinical presentation of patients with hemoglobinopathies attending an urban hospital in Ouagadougou: confirmation of the modification of the balance between HbS and Hb C in Burkina Faso**

**Simpore Jacques<sup>1</sup>, Pignatelli Salvatore<sup>1</sup>, Barlati Sergio<sup>2</sup> and Musumeci Salvatore<sup>3</sup>**

<sup>1</sup>Centre Medical Saint Camille (CMSC), Ouagadougou, Burkina Faso ;<sup>2</sup>Cattedra di Biologia e di Genetica, Università di Brescia, Italy, <sup>3</sup>Cattedra di Pediatria e Puericultura , Università di Sassari, Italy.

**Key words:** Hb S and Hb C phenotypes, Social and health changes, Burkina Faso

### **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 at the Laboratory of the Centre Medical Saint Camille (CMSC), Ouagadougou, Burkina Faso, for suspicion of haemoglobinopathy. 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 contrary the frequency of Hb CC is very low because it does not interfere with their health status. Moreover the high presence of Hb S 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 Centre to receive blood tests. The dramatic increase over the past few years is the prevalence of HbSS, who were absent in the study of Labie et al 1984 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 improved health and life expectancy.

Correspondence should be addressed to: Salvatore Musumeci, Paediatric Department, University of Sassari, Viale San Pietro n° 12, 07100 SASSARI, Italy. [smusumeci@tiscalinet.it](mailto:smusumeci@tiscalinet.it)

## 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 Falciparum* malaria in the younger years of life can not 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, also if the thromboembolic manifestations remain frequent. From data collected by Livingston in 1991 (2) seems evident that, in the last thirty years, a change in the health status of the different genotypes has taken place and therefore their prevalence 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 provide 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 part of the world.

## MATERIAL AND METHODS

### Samples

10,166 subjects, suspected of having a hemoglobinopathy, living in the urban area of Ouagadougou, were investigated in the course of 1997-99 at the Medical Centre Saint Camille (CMSC) of Ougadougou. 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 Centre 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 haemoglobin in the course of a health check-up, before starting a new work. Patients who were Hb SS homozygotes arrived at the CMSC because of a vascular-occlusive crisis and severe anaemia; patients compound heterozygotes for  $\beta$  C and  $\beta$  S were observed primarily for anaemia, but also for vascular occlusive pain.

## Methods

In all subjects, the screening was performed by Hb electrophoresis using cellulose acetate plates (Helena) with pH 8.6 buffer. When abnormal hemoglobins 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 a ADEL 16 (Minivolt) analyser. In 763 subjects, in addition to Hb electrophoresis, a complete evaluation of haematological parameters (RBC, Hb, Ht, MCH, MCHC, MCV, PLT, 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 from the real gene frequency 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 Hb SS was 9 years and the oldest patients observed were 22 years old. The other genotypes had a median age of 11 and the oldest of them was 42 years old and 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 thromboembolic manifestations and some also had severe anaemia, all were treated with aspirin treatment and hydrosaline infusion til symptoms resolved. Others came to the CMSC for pneumoniae or for degeneration of femur head.

52.5% of Hb SS subjects were diagnosed within the first 3 years of life, 30% between 4 and 15 years and 17.5% after 16 years. In the HbSC patients the diagnosis was made slight later and in a few occasions in adlthood, while in the patients with HbCC the diagnosis was made primarily when asymptomatic after 15 years on a routine health check (**Figure 1**). The number of episodes of thromboembolism per year in HbSS ranged between 2-24, with a median rate of 3 per year. The first manifestation in 30 % of children involved the metacarpal, metatarsal and proximal phalanges (the so called hand-foot syndome). The spleen is markedly enlarged in the first two years and later it became smaller due to repetitive infarctions.

70% of Hb SC patients showed rarer and less severe form of thromboembolism and a less prominent. On the other hand, they showed splenomegaly during the first five years of life. Infections were frequent in 55 % of Hb SS patients (pneumoniae and osteitis) and in 21 % of Hb SC patients. The patients who were homozygous for Hb CC were in good clinical conditions: 8% showed slight cutaneous jaundice, 17% had scleral jaundice and only 15% had splenomegaly of moderate degree.

**Table I** lists the haematological 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 Ht, while the MCV was reduced in the SC group and the MCHC was higher. Also PLT counts were higher in SC group, while the WBC count was higher in the CC group. Serum bilirubin (BT) was slightly elevated in HbSS and Hb CC, but only slight in Hb SC. In patients with Hb SS hydroxiurea (15-20 mg/Kg/day) and traditional drugs as FAGARA and DREPANOSTA were given occasionally to prevent the vascular occlusive crisis. Folic acid was prescribed to all subjects, who showed a low level of haemoglobin associated with the presence of Hb S and Hb C. Iron (1 mg/Kg/day) and folic acid (100 mg/day) were given to anaemic patients negative for haemoglobinopathies.

## DISCUSSION

The high number of homozygotes for  $\beta$  S detected in the Ouagadougou population (196/10166 corresponding to the 19.3 % of all subjects studied) as compared to the lack of cases detected in Labie et al, 1984 (5), suggests that the improved social and health conditions, the use of vaccines and the antibiotic prophylaxis for infective complications have played an important role in establishing a more balanced polymorphism with the Hb S–malaria relationship. Alouch, 1997 (6) have demonstrated that this relationship is maintained by a higher mortality rate of the Hb AAs due to malaria and the high mortality rate of Hb SSs and Hb SCs caused by complications of their disease, which are today better controlled than in the past.

The higher numbers of Hb SC (660) in this study are direct consequence of the selection process whereby patients presenting with anemia or pain due to thromboembolic 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  $\beta$  S gene in all countries of the Benin Gulf (7).

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 centre for testing. Indeed, the observation that Hb AS individuals are slightly anaemic, may be due to the younger age of this group, which was addressed to the CMSC as a consequence of expanded intrafamilial screening. Clinically the SS and SC patients show signs of chronic hemolysis and sickle cell disease (both thromboembolic 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 on  $\beta$  S is improved by the absence of associated pathologies (8). In fact Hb CC subjects observed at the CMSC, showed haematological parameters all within the normal range for the black

population, with the exception of a slight elevated total bilirubin. Recently Modiano et al (9) have demonstrated that Hb C provides protection against clinical *Plasmodium Falciparum* malaria in both the heterozygote (29%) and homozygote state (93%) and it represents the most important factor which maintains the  $\beta$  C gene frequency in Burkina Faso.

Life expectancy for patients with Hb SS in Africa can be improved by improving their clinical state. This can be achieved by introduction of new medical strategies such as hydroxiurea, hydration, aimed at reducing the frequency and severity of vascular occlusive crisis (10-11). The results of this study, which confirm the high rate of abnormal haemoglobins in Burkina Faso and their adverse effects on their health status in homozygotes and compound heterozygotes, focus on the need for secondary prevention. The development of sickle cell anaemia services, a problem not yet completely solved even in well-faring countries, becomes particularly important now that patients with sickle cell anaemia have a longer life expectancy.

### ACKNOWLEDGMENTS

The authors gratefully acknowledge the Department of Chemistry, University of Catania, for their hospitality in the late activities of the present study. Dr Dominique Labie is similarly acknowledged for her precious suggestions and constant help in the preparation of this paper. We thank the RADIM society (Italy), and remember with gratitude the late Dr. Franco SPARANO for his financial contribution. We are also deeply grateful to the CEI (Conférence Episcopale Italienne), for financing the project committed to the Laboratory of Centre Medical St Camille of Ouagadougou. Finally, all those who have helped and made this study feasible are gratefully thanked.

### REFERENCES

- 1) Serjeant GR.: Geography and the clinical picture of sickle cell disease. An overview. *Ann N Y Acad Sci.* **1989**;565:109-19.
- 2) Livingston RL.: Political interference with research? *JAMA.* **1991**; 266 (23): 3285-6
- 3) Devoucoux R, Hurpin C, Baudon D, Molez JF, Roux JF, Guilloud-Bataille M, Carnevale P, Feingold J: Population genetics of abnormal haemoglobins in Burkina Faso, West Africa. *Ann Hum Biol.* **1991**; 18(4):295-302
- 4) Sempore J, Pignatelli S, Barlati S, Musumeci S: Modification in the frequency of HbS and Hb C in Burkina Faso: an influence of migratory fluxes and improvement of patient health care. *Hemoglobin* **2002**, in press

- 5) Labie D, Richin C, Pagnier J, Gentilini M, Nagel R.L.: Hemoglobin S and C in Upper Volta. *Hum Genet* **1984**; 65: 300-302
- 6) Aluoch JR: Higher resistance to *Plasmodium falciparum* infection in patients with homozygous sickle cell disease in western Kenya. *Trop Med Int Health* **1997**; 2(6): 568-71
- 7) Friedman Mj, Roth EF, Nagel RL and Trager W: The role of Hemoglobin C , S, N-balt in the inhibition of malaria parasite development in vitro. *Am J Trop Med Hyg* **1979**; 28:777-780
- 8) Smith EW and Krevans JR: Clinical manifestations of hemoglobin C disorders. *Bull Johns Hopkins Hosp* **1959**; 104: 17-43
- 9) Modiano D, Luoni G, Sirina BS, Simpore J, Verra F, Konatè A, Rastelli E, Olivieri A, Calissano C, Paganotti GM, D'Urbano L, Sanou I, Sawadogo A, Modiano G and Coluzzi M. Haemoglobin C protects against clinical *Plasmodium falciparum* malaria. *Nature* **2001**; 414: 305-308.
- 10) Jardin F, Sane M, Cloatre G, Thiam M, Camara P, Pouolin S, M'Baye PS, Klotz F., Adults with sickle cell anemia in Senegal. Clinical study of 40 homozygote subjects. *Med Trop* **1999**; 59 (3): 271-5
- 11) Mouele R, Boukila V, Fourcade V, Feingold J, Galacteros F.: Sickle cell disease in Brazzaville, Congo: genetical, hematological, biochemical and clinical aspects. *Acta Haematol*; **1999**; 101 (4): 178-84

**Table I:** Haematological parameters of 763 studied subjects according the Hb genotypes

Hb Genotype	AA	AC	AS	CC	SC	SS
	(n°472)	(n°154)	(n°80)	(n°14)	(n°32)	(n°11)

---

RBC ( $10^{12}$ /L)	4.34±0.71	4.40±0.80	3.86±1.24*	4.31±0.48	4.27±0.45	3.15+/-0.80*
Hb (g/dl)	11.9±2.34	11.6±0.80	10.54±1.24*	11.03±0.48	10.90±0.45**	8,48+/-2.3*
Ht (%)	39.33±6.87	37.69±6.81	34.07±10.52	35.77±5.55	35.05±6.	25.0+/-9.6*
VCM (fl)	91.05±9.19	86.77±9.01*	90.05±7.19	83.15±9.83**	81.88±5.79*	85.8+/- 8.76
MCH (g/dl)	27.63±3.80	26.65±3.52	27.52±2.09	25.62±3.49	25.5±1.55	23.64+/-8.32*
MCHC (pg)	30.23±1.80	30.57±1.36	30.47±0.64	30.80±0.69	32.70±1.83*	31.42+/-1.85*
WBC ( $10^9$ /L)	6.31±2.89	7.16±3.64	9.44±8.20*	9.77±4.76*	10.50±4.38*	13.22+/-4.57*
PLT ( $10^9$ /L)	285±89	300±93.0	296±93	320±80	345±85*	262+/-52.6
BT (mg%)	0.80±0.45	0.93±0.66	0.85±0.41	1.52±0.79 *	1.25±0.63*	1.75+/-0.67*
BD (mg%)	0.40±0.23	0.54±0.22	0.35±0.24	0.76±0.46*	0.75±0.21*	0.80+/-0.35*
BI (mg%)	0.38±0.37	0.46±0.39	0.50±0.36	0.86±0.56*	0.50±0.42	0.95+/-0.34*

---

Student T test \* P< 0.0001; Student T test \*\* P< 0.001 Student T test \*\*\* P< 0.01

**Figure 1: Age at the diagnosis of haemoglobinopathy in the CMSC**





