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# Anthropological consideration on prevalence and fitness of β C and β S genotypes in Burkina Faso (a survey in the public schools).

We have studied To study the incidence of hemoglobinopathies (Hb C and Hb S) we have examined in 15,367 students, aged 11.4 +/- 4.64 years (median 11; range 1-26), living in Burkina Faso (12,019 were students of 23 public schools of Ouagadougou and 3348 students of 7 public schools situated in six villages about 12-35 Km from Ouagadougou).
In all groups studied, β S and β C gene frequencies were age dependent there was an age dependency of the β S and β C gene frequencies, since the advantage of HbS carriers in a malarial region is prevalently expressed in the first years of life. In fact, β C the gene frequency of β C increases, and the β S decreases with age. The Mossi, living prevalently mainly in Ouagadougou, show a gene frequency which is similar to the Bissa ethnic groups, where the C gene frequencies (0.116 and 0.118) wereare more higher than the S (0.049 and 0.044 respectively). On the contrary in the Peuhl ethnic group the β C and β S gene frequencies (0.049 and 0.049) were are the same, while in the Yorouba ethnic group immigrated from Nigeria a prevalence of β S gene frequency is higher (0.117) than over the β C (0.068)gene frequencies was found in the Yorouba ethnic group, who is immigrated from Nigeria, showing that different gene frequencies are found in different ethniae ethnic groupscorrespond to different gene frequencies.

*Key words:* Hb C, Hb S, schools, Burkina Faso, prevalence, fitness

## Introduction

Burkina Faso is today a country with 12 million people living in an area of 274,200 square kilometres. In recent years, migration from the countryside has increased to 27 % the urban population of the population live in urban centres, where an intense immigration process from the countryside has been registered in recent years. In factIndeed, in the period 1980-2000 the population of Ouagadougou has increased from 600,000 to 1,300,000. (1980-2000). The 73%The rest of the of population of Burkina Faso is distributed in 8,000 villages (Serjant, 1989). Thise features of this country, locked by the Niger River (once known as Upper Volta), is characterized by difficult a harsh environmental conditions, have and has maintained unchanged its genetic composition (see figure 1). The country is known for a high incidence of HbAC and HbAS carriers and for its *Plasmodium falciparum* malaria endemicity. The study by Labie study et al in 1984 demonstrated that the southern Savanna (a humid region with 900 mm

of rain per year), near Ouagadougou, inhabited specifically mainly by the Mossi, was characterised by higher β C (0.14) gene frequency of β C (0.14) over than β S (0.03); whereas, in the arid Sahel region of Sahel with rainfall of less than 500 mm a year, where, still today, a variety of ethnic groups live together (Songhraïs, Mellebe, Peuhl and Touaregs), the β S gene frequency of β S was notably higher (β S 0.1 vs β C 0,05) (Labie et al., 1984). These results confirm a possible negative correlation between the β C and β S gene frequencies of β C and β S, that is are at a significantly different level from that expected and localized the peak of β C gene frequency in the central region of Burkina Faso (Mossi plateau). Recently it has been demonstrated that Hb CC are less susceptible to malaria and this could explain the more elevatedhigher frequency of Hb C gene in regions where malaria in endemic (Modiano et al., 2001).

In the last thirty years, however, changes in the fitness of the different genotypes (AS individuals have a lower and AA and SS a higher fitness) have beenwere observed, which suggestsuggesting a partial relaxation due to a selection mechanism in this region (Livingston, 1991). An epidemiologicc survey, carried out in the schools of Ouagadougou and in the population of six villages near the town during 1999 - 2000 (Simpore et al., 2001) in publication), has demonstrateddemonstates an increase in the β S and a reduction in β C allele frequencies, probably due to changes in life expectancye of children with β C and β S phenotypes, but also to migration flows from the arid Sahel region of Sahel to the humid Savanna.

The aim of the present investigation is to evaluate, on a larger school population, the changes in the incidence of these hemoglobinopathies in Burkina Faso after the revolution of 1983, which has produced significant social and sanitary changes.


*Figure 1: Map of Ouagadougou indicating the location of the schoolsshowing schools location.*

## Materials and Methods

Samples:

In the period 2000-2001 we examined A total of 15367 students: were studied during 1999-2001. 12,019 were students of from 23 Ouagadougou public schoolsin Ouagadougou, 3348 were students attending from 7 schools of in six different villages (Boassa, GB Saaba, Koubri, Tanlarghin, Zagtouli, Ziniare) of Ouagadougou in the interland of Ouagadougou. The ratio female over male was 54.2 % were female and to 45.8 % male, their age was 11.4 +/- 4.64 (median 11; range 1-26) (see figure 2).

### Haemoglobin studies:

The blood was collected by finger puncture in a tube containing saline 0.9% NaCl, and the red cell were washed three time before the lysis. The hemolysate was analized with electrophoresis using cellulose acetate plates (Helena) with pH 8.6 buffer. When abnormal haemoglobins were was detected, citrate agar electrophoresis at pH 6,4 and a solubility test (to confirm the presence of Hb S and Hb C) was were performed. The percentage of abnormal Hb was evaluated by gel densitometry with the use of a ADEL 16 (Minivolt) analizer.

## Results

The results of Hb electrophoresis are shown in Table I A. Of the 15,367 subjects studied 5139 (33.44 %) had a β-chain mutation (C or S or both). Nobody had elevated A2 and the only pathological Hbs found at through the acetate cellulose electrophoresis were Hb S and Hb C. We found that 157 were heterozygoustes for both HbC and HbS, 256 were homozygouste for Hb C and 32 homozygouste for Hb S. The gene frequencies of β-chain mutations in Ougadougou schools and in the six villages are reported in Table I B. In the 23 Ouagadougou schoolsin Ouagadougou, a large variation in the alleles frequency β C (0.112 +/-0.015) and β S (0.049 +/- 0.012) was found. Similar variations were found in 7 schools of the villages (0.128 +/- 0.010 and 0.049 +/- 0.009 respectively) (see figure 3). Considering the geographical position of these villages with respect to the town of Ouagadougou, no significant differences were found in the β C and β S alleles frequencies. The observed incidence of CC and SS was found similar to that expectedin accordance with what expected from the Hardy Weinberg equation, but and only the incidence of SC incidence was found higher than expected in the village schools (table II). Evaluating the β C and β S gene frequencies of β C and β S according to sex, a prevalence of β C and β S ( 0.116 and 0.050) in males over females (0.113 and 0.047) was found in all schools (see Table II), with the only exception for of the Juvenat Garcon and Juvenat Filles where males and females constitute 100% respectively (in these two religious schools no subject with Hb SC and Hb SS was found because of preliminary selections). The cross tabulation of Hb electhrophoresis between the sexes also demonstrated a prevalence of females in the Hb SS group (65.5% female against 34.5% male) (figure 4).

Table III shows the β A, β S and β C gene frequencies of β A, β S and β C in all students studied for different age groups. We observed aA progressive reduction of A frequency and fluctuating values of of A frequency was observed β S frequency with age, whereas β C, ran-

**Table I:** Results of Hb electrophoresis (A) and frequencies of Hb alleles (B) concerning the two areas under study.

| Hb genotype | Ouagadougou Schools* (n. 12,019) | Village Schools ** (n. 3,348) | Total | **A** |
|---|---|---|---|---|
| AA | 8097 (67,3%) | 2131 (63.6%) | 10,228 (66.6%) | |
| AC | 2623 (21.82%) | 787 (23.5%) | 3,410 (22.2%) | |
| AS | 980 (8,15%) | 304 (9.08%) | 1,284 (8.4%) | |
| SC | 100 (0.83%) | 57 (1.70%) | 157 (1.0%) | |
| CC | 194 (1.61%) | 62 (1,85%) | 256 (1.7%) | |
| SS | 25 (0,208%) | 7 (0.209%) | 32 (0.2%) | |
| | | | total 15,367 | |

| Hb alleles | Ouagadougou Schools# | Village Schools ## | **B** |
|---|---|---|---|
| p β A | 0,824 | 0.800 | |
| q β S | 0,047 | 0.056 | |
| r β C | 0,129 | 0.144 | |
| β S + C | 0,176 | 0.200 | |

$X^2$ * →** P= 0.000; $X^2$ ## →### P= 0.100

**Table II:** Genotypic incidence of β chain mutation according to the places of investigation

| | Schools Ouagadougou | | Schools Villages | |
|---|---|---|---|---|
| Hb genotype | Ex° | Ob°° | Ex°°° | Ob°°°° |
| q2 SS | 0,0022 | 0.0020 | 0,0031 | 0.002 |
| | (26) | (25) | (10) | (7) |
| r2 CC | 0,0166 | 0.0161 | 0,0207 | 0.0185 |
| | (199) | (194) | (69) | (62) |
| 2qr SC | 0,0121 | 0.0166 | 0,0161 | 0.0170 |
| | (145) | (100) | (53) | (57) |

X2 °→°° P = =0.100; X2 °°° →°°°° P = 0.634

Ob= Observed frequencies Ex = Expected frequencies

**Table III:** Gene frequencies of hemoglobin S and C according to the age in the schools of Ouagadougou and in the schools of villages.

| Age group (years) | β A | β S | β C | total No genes |
|---|---|---|---|---|
| 1-5 years* | 0.846 (1140) | 0.045 (61) | 0.109 (147) | 1348 |
| 6-10 years** | 0.839 (10206) | 0.050 (569) | 0.111 (1345) | 12120 |
| 11-15 years*** | 0.838 (12372) | 0.047 (694) | 0.115 (1698) | 14764 |
| 16-20 years**** | 0.828 (1225) | 0.041 (61) | 0.131 (194) | 1480 |
| > 20 years ***** | 0.815 (831) | 0.059 (60) | 0.126 (128) | 1020 |

$X^2$ * →** P=0.935; $X^2$ ** → *** P=0.579; $X^2$ ***→**** P=0.127

$X^2$ ****→***** P=0.126

**Table IV:** Genotypic incidence of Hb SS, Hb SC, Hb CC in different age groups.

| Hb genotype | 1-5 years* | | 6-10 years** | | 11-15 years*** | | 16-20 years**** | | > 20 years***** | | total****** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ob | Ex | Ob | Ex | Ob | Ex | Ob | Ex | Ob | Ex | Ob | Ex |
| Hb SS | // | 2 | 22 | 15 | 6 | 16 | 1 | 1 | // | 2 | 29 (0.0019) | 36 (0.0023) |
| Hb SC | 10 | 9 | 66 | 67 | 70 | 80 | 10 | 8 | 6 | 6 | 162 (0.0105) | 170 (0.0110) |
| Hb CC | 8 | 10 | 69 | 75 | 118 | 98 | 9 | 13 | 7 | 8 | 211 (0.0137) | 204 (0.0132) |

Ob = frequency observed; Ex = frequency expected;

$X^2$ *Ob→*Ex P=0.358; $X^2$ **Ob→**Ex P= 0.453;

$X^2$ ***Ob→***Ex P=0.0.029; $X^2$ ****Ob→****Ex P=0.652

$X^2$ *****Ob→*****Ex P=0.412; $X^2$ ******Ob→******Ex P=0.611;

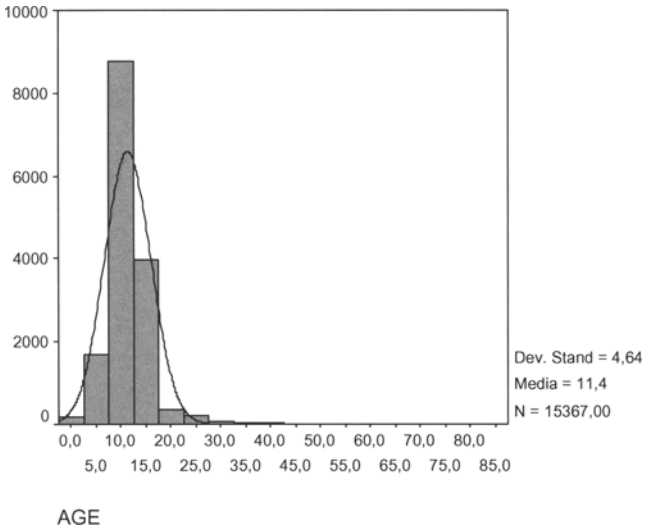


*Figure 2: Age Ddistribution for age of 15,367 students frequenting in 23 Ouagadougou schools and 7 village schools.*

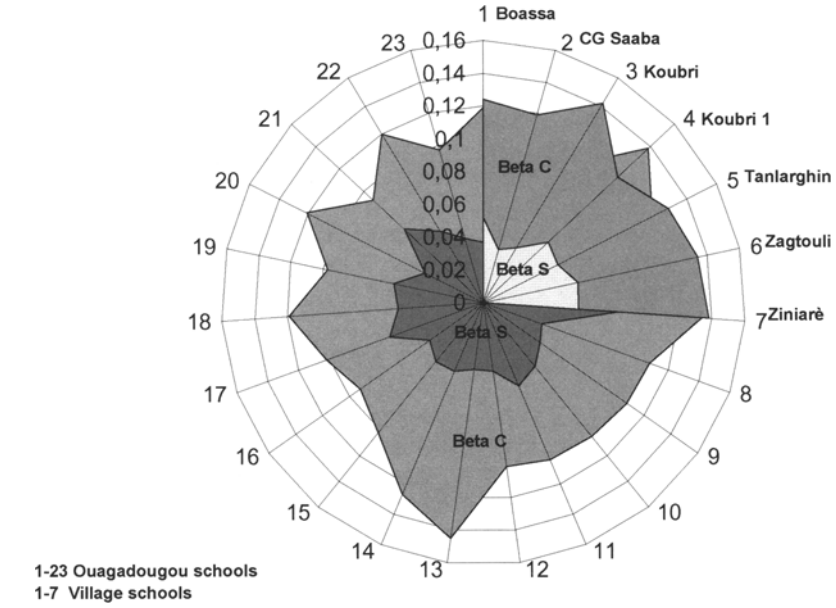


*Figure 3: C and S gene frequencies in Ouagadougou schools (1-23) and in village schools (1-7)*

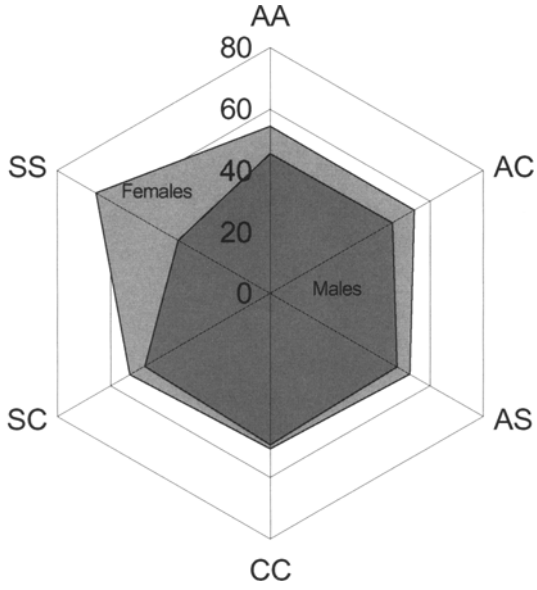


*Figure 4: Sex distribution of Hb genotypes in 15,367 students of Ouagadougou and village schools*

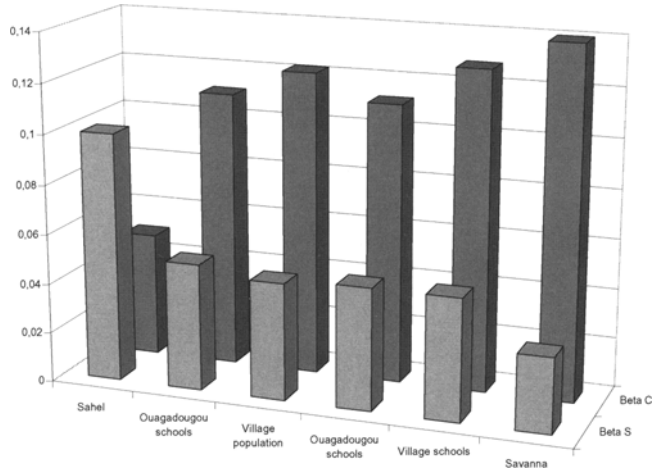


*Figure 5: Comparison of gene frequencies found in Ouagadougou and village schools (2000-2001) and in Ouagadougou population (1999-2000) with those found in Sahel and Savanna by Labie et al 1984.*

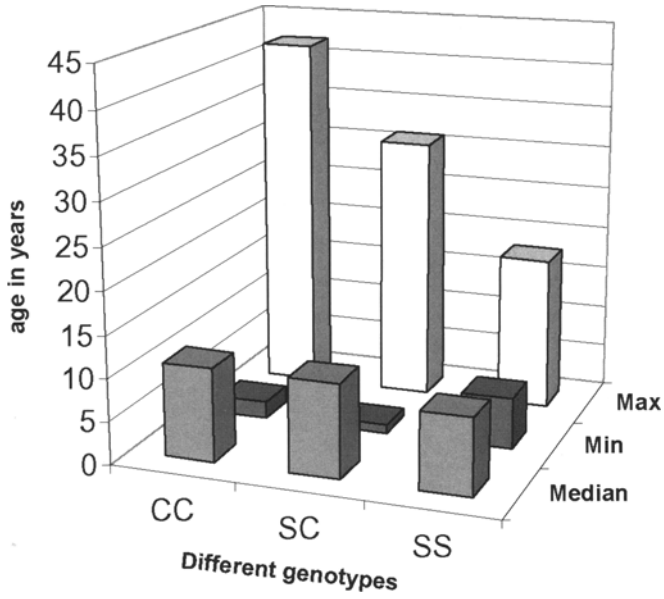


*Figure 6: Histogram of median ages and relative ranges in different Hb genotypes.*

ging from 0.108 to 0.131 the contrary was observed for S and C which showed variations ranging from 0.041 to 0.059 and from 0.108 to 0.131increased up to age 20. It is worth noticing that in all of Ouagadougou schools and in the six villages, the SS genotypes were less than expected in the 1-5, 10-15 and > 20 age groups; while in the 5-10 age group, the observed SS were higher than expected. The observed SC genotype was not statistically different from what the expected, while the observed CC genotype was more frequent in the 10-15 age group (see Table IV).

Correlating the gene frequencies in the various racesgroups, we found that the Mossi ethnic group, living prevalently mostly in Ouagadougou (14,354/15,367), showed a β C gene frequency of 0.116 and β S of 0.049 respectively. Also among theIn the Bissa ethnic group , we found a significant prevalence of β C (0.118)was found, while the incidence of S was 0.044. Conversely, the Peuhl ethnic group, living in the countryside and coaming from the Sahel, revealed a similar balanced gene frequency of for β C (0.049) and β S (0.049). Among the Yorouba ethnic group, immigrated from Nigeria, the gene frequency was for β C was 0.068 and for β S 0.117. In thehis latter ethnic group, the subjects affected by the Hb SC desease were 3.9%.

## Discussion

Of 15,367 subjects observed in Ouagadougou schools and in the six villages, 4,694 (30.6%) were heterozygous carriers of haemoglobin mutation, and 445 (2.89 %) were both homozygoustes or compound heterozygoustes. The calculated gene frequencies of β S (0.049 +/- 0.012) and β C (0.112 +/- 0.015) in schools, and of β S 0.049 +/- 0.009) and β C (0.128 +/- 0.010) in the villages resulted intermediate, from compared with those obtained in a previous study by Labie et al (1984) in the humid Savanna region, where a higher prevalence of β C (0.14) on β S (0.03) was found, in contrast with the prevalence of β S genes (0.105) on C (0.05) in the arid Sahel region (see figure 5). Two hypothesis could explain the higher percentage of βS found in Ouagadougou schools and in the six village schools of six villages near Ouagadougou: 1) an improved life expectancy of Hb SS and SC (see figure 6) might have increased the Hb S allele frequencyof Hb S allele. The Our rresults obtained in this survey, carried out in the schools, confirm suggest that the inverse correlation between these two S and C gene frequencies cannot be due considered only due to the different morbidity between of these two hemoglobinopathies at the heterozygote state, but more probably they appear tocan be a consequence of improved life expectancye of life of children with Hb SS and Hb SC in black Africa (Jardin et al., 1999). 2) a β S genes migration flow from the Sahel region of of S genes following the urbanization process of Ouagadougouafter the independence of 1960 from the poorer region of Sahel where a prevalence of β S genotype was demonstrated in the past to the richer Savanna region where Ouagadougou is situated (Labie et al., 1984); The differences found between the Mossi and Peuhl ethnic groups could also be due to the improved local customs of life, if the effect of migration flow cannot be excluded. In fact, the Peuhl ethnic group, who are shepherds and come from the Sahel and marry among cousins for two reasons: a) in order not to disperse family property, and b) since they are nomads, each family group wants to remain united, therefore they move and travel together. These are the two main reasons for consanguinity. Since Hb SS and Hb SC die at a very young age (Tattah and Ekere 1975), more children are born to compensate for their this loss, maintaeining the proportion between β S and β C in this ethnic groupniae. The contrary has been observed for the Mossi ethnic group which is similar to the Bissa. For a long time in the past, these two ethnic groups have been strictly connected and have maintained many customs in common, especially the habit of not marrying among cousins, since they consider their cousins as brothers (Hammond 2001). In this ethniae group the β C gene frequency is prevalent onhigher than the β S. The country of Bissa, near Ghana, is the focal point of HbC and from this part of West Africa this Hb diffonds. On the contrary, the elevated incidence of β S in the Yorouba ethnic group ( β S 0.117 over β C 0.068) is probably due to their origin from Nigeria where the Hb S is prevalent on Hb C (Kulkarni and Jekeme 1986).

The number of β S homozygous subjectstes for β S, in Ouagadougou schools (24/12,019 individuals, corresponding to the 0.2 % of subjects studied) and in the six villages (5/3,348 corresponding to the 0.2 % of subject studied), compared with the 1984 data (Labie et al., 1984), when any where no case of Hb SS was reported, suggests that the improved social and sanitary conditions, such as and the antimalaria and antibiotic prophylaxis, have certainly played an important role in establishing a more balanced Hb S–malaria relationship, which is maintained by a higher mortality risk of Hb AAs due to malaria and a high mortality risk of Hb SSs and Hb SCs caused by infectiousve complications (Aluoch 1967). As a direct consequence of improved medical assistance, today more students affected by Hb SC and Hb SS can attend school and aspire to a healthier life. Therefore, an early detection of subjects affected by Hb

SS and Hb SC in the first years of life is very important, for the control of these severe haemoglobinopathies (Brown et al 1994).

The higher proportion of β S alleles (0.050) in the first 10 years of life of school children in Ouagadougou and the six villages could be explained, according to Luzzatto, 1979, by the fact that the advantage of Hb S carriers in a malarial region is expressed early, before immunological defences are built. In fact several observations suggest that the sickle cell trait does not prevent malaria infection (Chippaux et al., 1992; Wurie et al., 1996), but it is effective in protecting from severe and often fatal attacks of *Plasmodium falciparum* cerebral or hepatic malaria, which are frequent during infancy (Gendrel et al., 1992).

Another point of consideration is the constant increase with the age (from 0.109 to 0.131) of the β C gene frequency in the schools of Ouagadougou and neighbouring villages, which could be similarly explained by the advantage of heterozygosity te over the malaria parasite. A study case-control study in among the Dogons living in Bandiagara, Mali, has demonstrated that Hb C can protect against severe malaria, not against infection or uncomplicated malaria (Agarwal et al., 2000). Recently Modiano et al., 2001 have demonstrated that Hb C provides protection against clinical *Plasmodium Falciparum* malaria in both the hetherozygousote (29%) and homozygouste state (93%) and it represents the most important factore which mainteinmantainig the C gene frequency in Burkina Faso. The results of this survey, suggest not only that a protective effect against malaria is associated with Hb C, but that it is greater than Hb S in that part of West Africa. In fact if the life conditions of homozygous Hb CC are absolutely compatible with ordinary activities, including the reproductive capacity, the advantage of β C on β S may be further improved by the absence of associated pathologies (Smith and Krevans 1959). The observed genotype incidence of Hb CC in all the schools is closer (0.0161) to the expected one (0.0166). From an epidemiological point of view, also Hb SC individuals have contributed to the permanence and expansion of β S and β C genes in all countries of the Benin Gulf, since their life expectancy is better than that of SS and possibly also through some resistance to malaria of heterozygouste for both S and C (Friedman et al 1979).

If survival is improved after the 1983, also the clinical status of patients affected by Hb SS and Hb SC is was also improved in this part of Africa (Jardin et al., 1999; Mouele et al., 1999), with the introduction of Hydroxiurea (Ohene-Frempong and Smith-Whitley, 1997) and some empiric and traditional medicaments (FAGARA, DREPANOSTA) (Thiam et al 1990), aimed at reducing the frequency and severity of vascular occlusive crisis. In the present reality, since patients with sickle cell anaemia have a longer life expectancy, it becomes particularly important to develop sickle cell anemia services, together with a wider distribution of drugs for the prevention of sickling crises. Moreover, the adverse consequences on health the state ofstatus health of these hemoglobinopathies, now that the affected children affected have a longer life expectancye, suggest an urgent need of specialistic dispensaries for primary and secondary prevention, in order to identify Hb S carriers and provide adequate genetic counselling.

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