Admission of Hb S heterozygotes to a general hospital is relatively reduced in malarial areas

B COLOMBO* AND L FELICETTI

From Maputo Central Hospital, Maputo, Mozambique; and Institute of Cell Biology, Via Romagnosi 18/A, 00196 Rome, Italy.

SUMMARY A comparison between the frequency of Hb S heterozygotes in blood donors, outpatients, and inpatients of a general hospital carried out at the Maputo Central Hospital, Mozambique, where *Plasmodium falciparum* malaria is endemic, showed a statistically significant lower percentage of Hb S heterozygotes in the inpatient group. Evidence is thus provided that the protection given by Hb S to heterozygotes concerns not only malarial infection itself, but probably a wide spectrum of diseases to which persons who have a special resistance to *P falciparum* infection are less prone.

It is now well established that malaria has been acting as an evolutionary agent which selected several specific resistant genes in regions where the disease is endemic. Conclusive information is available for several genes expressed in erythrocytes, such as the β^s gene, some genes associated with G6PD deficiency, and with the Duffy blood group system.¹ The protective effect of Hb S in heterozygotes has been shown by a variety of observations. Correlations between the epidemiology of malaria and the prevalence of the β^s gene have been found even within restricted geographical areas (micro-mapping). For example, in the Ewe ethnic group in Togo, the frequency of the β^s gene in people living at low altitude is five-fold higher than that found in people living at high altitude.² Moreover, a high resistance of Hb S carriers to *P falciparum* has been indirectly demonstrated by the relatively low incidence of Hb S heterozygotes among patients with the most severe forms of malaria infection, and directly confirmed by experiments in volunteers inoculated with the parasite.³ Finally, the mechanism by which Hb S protects against *P falciparum* infection has been elucidated.^{4,5}

In endemic areas the fitness of Hb S heterozygotes, compared to that of Hb A homozygotes, has been estimated to be about 10% higher, and

perhaps in some cases up to 25% higher.⁶ The question then arises whether this selective advantage is due only to the direct protection from malaria infection, or also to an indirect protection against other diseases, the frequency of which is increased by previous malaria infection. Although various sequelae and a higher susceptibility to certain diseases have been demonstrated in recurrent malaria,⁷ no statistical data are available on the secondary effects of *P falciparum* infection in a population.

We obtained information on this question by comparing the frequency of Hb S heterozygotes in blood donors, outpatients, and inpatients of a general hospital in Maputo, Mozambique, where *P falciparum* malaria is endemic.

Patients and methods

Three groups of subjects were studied during a period of nine months. The first group consisted of 4763 blood donors of the Maputo Blood Bank, the second of 5214 outpatients of the Maputo Central Hospital, and the third of 4648 inpatients from all the divisions of the same Hospital. The age composition of the inpatients and outpatients was similar; in these two groups all ages were represented, while in the blood donors ages ranged from 18 to 46 years. The inpatient group did not include subjects affected only by malaria, since they are treated in other centres.

Haemoglobin electrophoresis was carried out on

*Present address: Institute of Cell Biology, Via Romagnosi 18/A, 00196 Rome, Italy.

Received for publication 3 August 1984.

Accepted for publication 7 September 1984.

cellulose acetate (Beckman Microzone Electrophoresis membranes) in a TRIS-EDTA-borate buffer at pH 8.6.⁸

All samples showing an abnormal electrophoretic band were submitted to the dithionite solubility test in a concentrated phosphate buffer.⁹

Results and discussion

The results of the screening are shown in table 1. It can be seen that the frequency of Hb S heterozygotes in the blood donors and in the outpatients was quite similar (0.99 ± 0.14 and 1.13 ± 0.15 respectively). These figures are in agreement with previous studies, which also show that the frequency of the β^S gene is homogeneous in the different ethnic groups living in southern Mozambique.¹⁰

On the other hand, the inpatient group showed a significantly lower frequency of heterozygotes (0.65 ± 0.12). This difference is not found in regions where malaria is absent. In table 2 we show a compilation of pertinent data by ourselves and by other authors in different geographical areas which demonstrate that in non-malarial areas the frequency of Hb S heterozygotes among people admitted to hospital is not lower than in the normal population.

On the basis of the above findings we suggest that in the presence of endemic malaria, Hb S heterozygotes are approximately 35% less susceptible than Hb A homozygotes to different diseases which require admission to hospital.

It would be of interest to repeat the same analysis in malarial regions where the frequency of the β^S gene is higher.

TABLE 1 Frequency of Hb S heterozygotes in the three groups studied at the Maputo Central Hospital, Mozambique.

	No of subjects	No of Hb S heterozygotes	% Hb S heterozygotes
1 Blood donors	4763	47	0.99 ± 0.14
2 Outpatients	5214	59	1.13 ± 0.15
3 Inpatients	4648	30	0.65 ± 0.12

1 vs 2 $p > 0.4$; 1 vs 3 $p < 0.1$; 2 vs 3 $p = 0.01$; 1+2 vs 3 $p < 0.02$.

TABLE 2 Comparison of Hb S heterozygote frequency in normal subjects and those admitted to hospital in areas where malaria is absent.

Place	Inpatients			Normal population		
	No tested	% Hb S heterozygotes	Ref	No tested	% Hb S heterozygotes	Ref
Dallas (USA)	1385	7	11	1165	6.7	6
Memphis (USA)	1413	8.8	12	2011	7.9	13
Porto Alegre (Brazil)	615	6.2	14	399	5.5	14
Havana (Cuba)	6447	3.08	15	24 458	3.12	16

The authors are indebted to Professors Lucio Luzzatto and Guido Modiano for helpful discussions and for revising the manuscript.

References

- Luzzatto L. Genetics of red cells and susceptibility to malaria. *Blood* 1979;54:961-76.
- Bienze U, Okoye VCN, Goegler H. Haemoglobin and glucose-6-phosphate dehydrogenase variants: distribution in relation to malaria endemicity in a Togolese population. *Z Tropenmed Parasitol* 1972;23:56-61.
- Livingstone FB. Malaria and human polymorphism. *Ann Rev Genet* 1971;5:33-64.
- Luzzatto L, Nwachukwu-Jarrett ES, Reddy S. Increased sickling of parasitized erythrocytes as mechanisms of resistance against malaria in the sickle-cell trait. *Lancet* 1970;i:319-21.
- Roth EF, Friedman M, Ueda Y, Tellez I, Trager W, Nagel RL. Sickling rates of human AS red cells infected in vitro with *Plasmodium falciparum* malaria. *Science* 1978;202:650-2.
- Livingstone FB. *Abnormal hemoglobin in human populations*. Chicago: Aldine, 1967.
- Manson-Bahr PEC, Apted FIC. *Manson's tropical diseases*. London: Balliere-Tindall, 1982.
- Smithies O. An improved procedure for starch gel electrophoresis: further variations in the serum proteins of normal human adults. *Biochem J* 1959;71:585-7.
- Nalbandian RM, Nichols BM, Camp FR, et al. The dithionite tube test, a rapid, inexpensive technique for the detection of Hb S and non-S sickling hemoglobins. *Clin Chem* 1971;17:1028-32.
- Nunes de Almeida R. Hemoglobinas anormais em Moçambique. *Est Gerais Universidade Moçambique*, 1967;IV,serie III: 95-107.
- Long WK, Wilson SW, Frenkel EP. Associations between red cell G-6-PD variants and vascular diseases. *Am J Hum Genet* 1967;19:35-53.
- McCormick WF, Kashgarian M. Abnormal hemoglobins. V. Age at death of patients with sickle-cell trait. *Am J Hum Genet* 1965;17:101-3.
- Adams JQ, Withacre FE, Diggs LW. Pregnancy and sickle-cell disease. *Obstet Gynecol* 1953;2:335-52.
- Tondo CV, Salzano F. Abnormal hemoglobins in a Brazilian negro population. *Am J Hum Genet* 1962;14:401-6.
- Vidal H, Hernandez A, Colombo B. Genetic and clinical relevance of haemoglobin screening: results from a survey in a paediatric hospital. *Clin Genet* 1974;5:31-5.
- Herederer L, Granda H, Altland K. A procedure of hemoglobin S screening for the genetic consultation in Cuba. Analysis of 24,000 blood samples. *Genetika* 1978;14:1079-84.

Correspondence and requests for reprints to Dr B Colombo, Institute of Cell Biology, Via Romagnosi 18/A, 00196 Rome, Italy.