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Malaria, cause of ahaptoglobinaemia in Africans

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Abstract

The lack of serum haptoglobin in Africans has been investigated in the Congo, Central Africa, where HpO prevalence is about 30%. This study shows that it is possible to suppress ahaptoglobinaemia within a few weeks by antimalarial chemoprophylaxis, that it does not occur in protected individuals, that ahaptoglobinaemia reappears at its original incidence levels after interruption of chemoprophylaxis, and that some individuals are more susceptible in relation to Hp^2 gene. Malaria is the only significant cause of ahaptoglobinaemia in subjects both with and without detectable parasitaemia. The possible mechanisms involved are discussed.

Introduction

The cause of the lack of serum haptoglobin in Africans, first reported in Nigerians by ALLISON *et al.* (1958), remains unknown. About 20 to 40% of individuals are ahaptoglobinaemic (HpO) in tropical Africa (ALLISON *et al.*, 1958; BLUMBERG & GENTILE, 1961; GIBLETT *et al.*, 1966; SUTTON, 1970; ROUGEMONT *et al.*, 1974; BOREHAM *et al.*, 1981). Elsewhere, HpO is rare (less than 1% of individuals) except in American Negroes (about 4%) and in some tropical forest populations (GIBLETT, 1959, 1969; SUTTON *et al.*, 1960; CURTAIN *et al.*, 1965; SUTTON, 1970). It has been suggested by ALLISON *et al.*, 1958, ALLISON, 1959, GIBLETT & STEINBERG, 1960, PARKER & BEARN, 1963, and VU TIEN *et al.*, 1975a and b that genetic factors, and by CURTAIN *et al.*, 1965, GIBLETT *et al.*, 1966, SUTTON, 1970 and LEFEVRE-WITIER, 1974 that environmental factors play roles in its aetiology. In Africans, a significant association between malaria parasitaemia and HpO has been reported (ROUGEMONT *et al.*, 1974; WELCH *et al.*, 1979; BOREHAM *et al.*, 1981; MONJOUR *et al.*, 1982). However, there has been no clear evidence that malaria was the only significant factor involved. Here we report the results of a longitudinal study made on schoolchildren in Linzolo, Congo, where HpO prevalence is about 30%.

Methods and Results

From November 1980 to January 1982, 14 successive surveys which included a battery of biological and clinical investigations were made on 250 schoolchildren in Linzolo village, Congo, 25 km south-west of Brazzaville. Plasma samples were collected by finger prick. The Hp level was measured by immunonephelometry. Plasma was diluted 1/200 in 9% NaCl solution. Anti-Hp serum, diluted 1/80, was then added. The antigen-antibody complexes were measured by a Technicon fluonephelometer. This method can detect Hp levels as low as 5 mg/100 ml.

HpO prevalence and incidence

The first three surveys were made at intervals of two months and 163 schoolchildren were present in all three. The number of HpO individuals was respectively 54 (33·1%), 51 (31·3%) and 49 (30·1%). In most cases ahaptoglobinaemia was a transient finding: in half of the individuals found HpO in one survey, haptoglobin was found at the next. Only 18 individuals (11%) remained constantly HpO in all three surveys (Table I).

Correlations with malaria parasitaemia

The examination of thick smears showed a correlation between the frequency of HpO and malaria parasitaemia. The mean parasite rate was 78·6%. The proportion of HpO individuals was 20·2% in those cases with a negative thick smear but reached 42·1% in those heavily infected (Table II). With reference to species of *Plasmodium*, the HpO frequency was highest (45·4%) in cases of infection with *P.*

Table I—HpO appearance and disappearance in the same subjects in the course of three surveys made at intervals of two months

	Surveys compared								
Surveys	1	2	2	3	1	3	1	2	3
No. of subjects	226		213		204		163		
No. of HpO subjects	72 (31·9%)	57 (25·2%)	63 (29·6%)	65 (30·5%)	65 (31·9%)	60 (29·4%)	54 (33·1%)	51 (31·3%)	49 (30·1%)
No. of subjects with temporary HpO	44 (19·5%)	29 (12·8%)	23 (10·8%)	25 (11·7%)	34 (16·7%)	29 (14·2%)	36 (22·1%)	33 (20·2%)	31 (19%)
No. of subjects with constant HpO	28 (12·4%)		40 (18·8%)		31 (15·2%)		18 (11%)		

Table II—Percentage of HpO according to malaria parasitaemia (All species; surveys 1, 2, 3 and November 81). Parasitaemia was assessed by a standard examination of 200 fields of stained thick blood film and parasite count in relation to the number of leucocytes on the basis of 8000 per μl of blood. Parasite density classes: 0: no parasite observed; 1: <50/μl; 2: 50-499/μl; 3: 500-4999/μl; 4: 5000/49999/μl; 5: ≥50000/μl. A single parasite observed in 200 fields corresponds to about 2·5 parasites per μl

Parasite density (classes)	Hp present	HpO	% HpO	No.
0	142	36	20·2%	178
1	114	35	23·5%	149
2	132	65	33%	197
3	103	62	37·6%	165
4	73	53	42·1%	126
5	11	4	26·7%	15
Total	575	255	30·7%	830

Table III—Percentage of HpO to *Plasmodium malariae* parasite density (surveys 1, 2, 3 and November 81)

Parasite density (classes)	Hp present	HpO	% HpO	No.
1	28	19	40·4%	47
2	24	19	44·2%	43
3	7	11	61·1%	18
Total	59	49	45·4%	108

Table IV—*Plasmodium malariae* infections: associated *P. falciparum* parasitaemia according to the presence or absence of haptoglobin

	P. falciparum parasite density						
	0	1	2	3	4	5	Total
Hp present	9	4	16	18	12	0	59
HpO	8	2	10	17	12	0	49
Total	17	6	26	35	24	0	108

malariae, despite a generally low parasitaemia, and reached 61·1% in heavy infections with this species (Tables III and IV).

Effect of amodiaquine

After the third survey, weekly antimalarial chemoprophylaxis with amodiaquine (Flavoquine^{R}, 10 mg/kg) was started in 167 schoolchildren for three months (April to June 1981). At the beginning of treatment, 55 schoolchildren (32·9%) were HpO. After six weeks, only four of 164 (2·4%) schoolchildren and after three months, only two of 119 (1·7%) schoolchildren were HpO. All the individuals absent at this last survey were Hp positive at the previous survey.

During chemoprophylaxis, seven additional weekly surveys were made in 54 individuals, of whom 33 were HpO at the beginning of treatment. Haptoglobin appeared in them after a delay of one week to two months and usually in less than one month. However, a transient ahaptoglobinaemia was observed in seven individuals under treatment. Six of these individuals (Table V: subjects Nos. 311, 315, 327 and 353; Table VI: subjects Nos. 251 and 542) were already HpO at the beginning of treatment: after generally appearing rapidly, usually within less than one month, haptoglobin disappeared before reappearing most often at the end of the second month and remaining in three or four successive weekly tests.

17 of the 18 individuals who were constantly HpO in the first three surveys were included in this study. All standardized their haptoglobin level when treated with amodiaquine (Table V).

Results after the interruption of amodiaquine

Chemoprophylaxis was interrupted during five months (July to November 1981). A fresh survey was made in November. 104 of the schoolchildren who had previously had chemoprophylaxis were found. 34 (32·7%) were HpO in April at the beginning of chemoprophylaxis and all had standardized their haptoglobin level when treated. In November, 27 (26%) 16 of which were new individuals were HpO.

This survey also included 126 subjects who had not previously had chemoprophylaxis: 32 subjects (25·4%) were HpO. Among the 126 subjects, 59 had been previously investigated in April and 14 (23·7%) were HpO at that time; in November, 13 subjects (22%) were HpO, but only four of these were the same individuals as in April.

Table V—Evolution of haptoglobin level (mg/100 ml) under weekly antimalarial chemoprophylaxis with amodiaquine in 17 schoolchildren who were constantly HpO in the first three surveys

Patient	Days									
	0	8	15	25	40	49	61	69	75	82
217	0	—	—	—	35	—	—	—	—	—
248	0	55	—	145	110	210	200	200	145	130
311	0	0	100	—	0	0	45	185	55	50
315	0	0	110	0	45	275	60	185	110	145
327	0	0	25	0	0	0	40	60	65	75
343	0	—	—	35	45	100	75	120	50	—
346	0	—	—	—	100	—	125	265	145	—
353	0	—	—	10	20	0	40	55	45	—
365	0	—	—	—	—	—	40	65	—	—
372	0	—	—	—	130	—	125	310	130	110
375	0	—	—	—	65	—	110	175	165	220
381	0	30	120	110	110	185	190	310	120	—
385	0	—	—	—	145	—	175	330	—	—
388	0	0	—	—	65	90	155	155	130	165
390	0	—	—	—	45	—	55	80	55	75
391	0	0	—	—	35	65	90	175	130	145
405	0	—	—	—	0	—	120	—	—	100

Table VI—Evolution of haptoglobin level (mg/100 ml) under weekly antimalarial chemoprophylaxis with amodiaquine in 16 schoolchildren who were HpO at the beginning of the treatment

Subject	Days									
	0	8	15	25	40	49	61	69	75	82
251	0	—	—	—	145	0	200	155	90	100
267	0	55	110	105	50	50	175	200	145	130
277	0	25	—	90	110	115	120	145	45	—
278	0	65	175	210	105	185	190	275	110	50
291	0	—	—	120	130	—	130	—	—	120
301	0	0	130	80	95	100	90	155	145	110
309	0	—	—	—	65	—	120	—	90	275
319	0	0	90	—	50	110	175	275	130	120
330	0	0	80	145	165	295	145	155	75	45
333	0	90	100	135	100	130	330	250	175	275
339	0	55	—	130	90	155	165	240	110	—
348	0	—	165	—	120	—	—	250	165	—
407	0	65	145	110	90	115	145	175	110	100
412	0	—	—	50	55	—	145	240	110	265
508	0	—	—	—	90	—	120	—	100	25
542	0	—	—	—	25	—	45	65	35	0

Table VII—Distribution of haptoglobin types in (A) 32 individuals who had never been HpO in four samples taken in the absence of chemoprophylaxis and (B) 18 individuals who have been at least three times HpO in four samples taken under the same conditions. The samples were collected at a minimum interval of two months

Group	No.	Phenotype			Hp^2 frequency
		1-1	2-1	2-2	
A	32	14	17	1	0·30
B	18	2	13	3	0·53

Table VIII—Distribution of HbAS individuals according to the number of times HpO was observed in the same individual in the course of three surveys made at intervals of two months

HpO	0	1	2	3	Total
No. of individuals (Total)	73	43	29	18	163
No. of individuals with HbAS	20 (27·4%)	6 (13·9%)	6 (20·7%)	9 (50%)	41 (25·2%)

Comparison of amodiaquine and mefloquine

The 174 schoolchildren were randomly divided into two groups. One group of 89 was treated weekly with amodiaquine as previously, and the other (85) with a monthly dose of mefloquine (20 mg/kg up to 25 kg, 500 mg from 25 to 40 kg, 625 mg above 40 kg). After two months (January 1982), another survey was done on both groups. Of the 89 individuals treated with amodiaquine, none was HpO, compared with 29 (32·6%) who had been HpO at the beginning of treatment. Of the 85 individuals treated with mefloquine, only one was HpO (1·2%), whereas 22 (25·9%) had been HpO at the beginning of treatment.

Correlations with haptoglobin phenotype

The haptoglobin phenotype was determined by the polyacrylamide gradient gel method (ENGLER *et al.*, 1973) in (a) 32 individuals who had never been HpO in the four samples taken in the absence of chemoprophylaxis and (b) 18 individuals who were HpO at least three times in the same four samples. The three common phenotypes were observed in the two groups of individuals (Table VII). However the Hp^2 gene frequency was significantly higher ($P<0·05$) in those individuals who were regularly HpO.

Correlations with sickle cell trait

Sickle cell trait (HbAS) was observed in 41 of the 163 schoolchildren present in the first three surveys.

To investigate if individuals with HbAS were more frequently HpO than others, we studied their distribution according to the number of times HpO occurred in the same individual in the three surveys.

Results are given in Table VIII. There appears to be no relation between sickle cell trait and HpO. However, 9 of the 18 individuals constantly HpO in these three surveys are HbAS subjects.

Discussion

Our results show that it is possible to eliminate ahaptoglobinaemia in a population in tropical Africa through antimalarial chemoprophylaxis. Of the 237 schoolchildren studied while receiving chemoprophylaxis, not one remained constantly HpO. The existence of a null allele Hp^0, which has been proposed for a long time to explain the high frequency of HpO in Africans, can be excluded in this population. Potential causes of ahaptoglobinaemia other than malaria,

whether genetic or acquired, can be excluded as significant causes and their frequency is no higher than that observed in the non-African populations.

How does malaria act? The function of haptoglobin is to bind free haemoglobin. It is probable that when infected erythrocytes are haemolysed, both by direct damage by invasion and growth of the parasites and by sequestration of parasitized cells in the spleen and other parts of the micro-circulation, haemoglobin is liberated and swiftly complexed with haptoglobin, thus initiating a fall in Hp level correlated with the severity of the parasitaemia. However, HpO was frequently observed in individuals with negative thick smears or scanty parasitaemia. The reappearance of haptoglobin in these cases when given antimalarials clearly shows that malaria is responsible. A possible explanation is that the level of parasitaemia can vary greatly, from very low to very high within a few days; it is thus possible that a simple analysis of parasite density as detected by examination of one blood film is inadequate to reveal a consistent, quantitative relationship of malaria to HpO.

However, there is evidence which suggests that mechanisms other than haemolysis of infected red cells are involved: Firstly, previous studies made in non-immune patients with malaria did not show a complete disappearance of haptoglobin, but only a decrease in Hp level (BLUMBERG *et al.*, 1963; AREEKUL *et al.*, 1972; PATWARI *et al.*, 1979); although ahaptoglobinaemia can certainly occur in this type of patient, these observations are all the more significant as the parasite density observed in non-immune individuals is generally high. Secondly, surveys conducted in adults in Linzolo show that HpO is frequent despite very low parasite densities. Among 154 adults over 40 years old investigated, HpO was observed in 22 (14·3%). Thick smears from 102 of these adults (14 of whom were HpO), showed no parasite or a parasitaemia lower than 50/μl in 84·3% of cases, and the maximum parasitaemia observed was 550/μl. Furthermore, investigations in febrile adults living in neighbouring villages showed a maximum parasitaemia of 600/μl in those over 40 years old. Higher levels of parasitaemia were observed in more than 40% of schoolchildren in each cross sectional survey.

Finally, our data showed that even in children receiving continuous chemoprophylaxis, haptoglobin concentrations fluctuated markedly and, in some cases, transiently totally disappeared. These fluctuations were observed in the absence of recurrence of parasitaemia and agree with previous observations by BOREHAM *et al.* (1981) who used pyrimethamine as a chemoprophylactic. Boreham suggested that HpO in such cases was due to factors other than malaria. Our data clearly show that cases of transient HpO were frequent in the first weeks of chemoprophylaxis but very rare after the second month (only one case among 110 tests performed between days 61 and 82 after the beginning of chemoprophylaxis in subjects previously HpO). This suggests that no factor other than malaria was involved in most of these cases.

Mechanisms other than the destruction of parasitized red cells that may be involved concern haptoglobin synthesis and the immune destruction of red cells.

A decrease in the synthesis of haptoglobin resulting from malaria infection would account for a persistent ahaptoglobinaemia once parasitaemia has disappeared. However, this seems to be very unlikely since an increase of haptoglobin synthesis is observed in any inflammatory syndrome which is not associated with liver alterations (ENGLER & JAYLE, 1976). A significant increase of the mean level of α_1 glycoprotein (orosomucoïd) was observed in HpO individuals, which paradoxically suggests an increase in haptoglobin synthesis. This would constitute a mechanism which opposes ahaptoglobinaemia.

The hypothesis which would account best for our observations is that of the existence in HpO individuals of a high auto-immune haemolysis induced by malaria. It has been shown that immune complexes fixed on to the surface of non parasitized erythrocytes are responsible for a persistent haemolysis several weeks after the disappearance of parasitaemia (ROSENBERG *et al.*, 1973; WOODRUFF *et al.*, 1979). Studies in the Gambia showed that red cell sensitization occurs frequently in *P. falciparum* malaria in individuals living in hyperendemic areas (FACER *et al.*, 1979; ABDALLA *et al.*, 1980; FACER, 1980; ABDALLA & WEATHERALL, 1982). However, these studies could not adduce convincing evidence that erythrocyte sensitization was frequently associated with enhanced red cell destruction (WEATHERALL & ABDALLA, 1982).

Genetic factors have been thought to play an essential role for a long time. Our study shows that the determination of HpO in tropical African populations cannot be explained on a direct genetic basis. Of the 237 schoolchildren investigated while receiving chemoprophylaxis not one remained constantly HpO. The existence of a null allele Hp^0 can be excluded in this population. However, the difference of Hp^2 gene frequencies in the two subgroups of subjects found HpO at least three times or never found HpO indicate a greater susceptibility to the "suppressive" effect of haemolysis in relation to Hp^2 gene. It is known that the haptoglobin binding capacity is phenotype dependent (CURTAIN *et al.*, 1965; SUTTON, 1970). Since types 2-2 and 2-1 haptoglobins bind less haemoglobin than type 1-1, one would expect to find types 2-2 and 2-1 individuals ahaptoglobinaemic at levels of haemolysis which still leave type 1-1 individuals with detectable haptoglobin. This explains the apparent relative inheritance of HpO found in many studies. Furthermore, this leads to a re-evaluation of many of the Hp^1 and Hp^2 gene frequencies reported from tropical Africa.

Conclusion

Although it clearly appears that malaria is the only significant cause of ahaptoglobinaemia in tropical African populations, the mechanisms are undoubtedly multifactorial and reflect an extremely complex series of interactions. The following factors which are certainly involved include: (i) haemolysis of parasitized red cells, (ii) plasmodial species, (iii) immune status of the subject and (iv) haptoglobin phenotype. Factors which are probably involved are: (v) immune destruction of red cells and (vi) enhanced synthesis of haptoglobin in response to malarial infection.

There is indirect evidence that the immune destruction of red cells contributes significantly to HpO. This suggests the existence of an important immune component of the anaemia of malaria in semi-immune

populations, although repeated recent attempts to demonstrate it were largely unsuccessful.

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