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AHAPTOGLOBINEMIA IN AFRICAN POPULATIONS AND ITS RELATION TO MALARIA ENDEMICITY

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A study of the relations between plasma haptoglobin levels and malaria endemicity was carried out on selected specimens collected in 1980–1985 during studies on malaria transmission in various populations of the Brazzaville region of the Republic of the Congo. The prevalence of ahaptoglobinemia in schoolchildren is 2.2% in Moungali and 2.9% in Poto-Poto, two districts of Brazzaville where malaria transmission intensity is less than one infective mosquito bite per person per year and malaria prevalence in schoolchildren is less than 10%. In contrast, ahaptoglobinemia prevalence is 48% in schoolchildren from the village of Djoumouna, where malaria transmission intensity reaches 1,000 infective bites per person per year and malaria prevalence in schoolchildren is 94%. Intermediate values, between 11.1% and 23.4% are observed in schoolchildren from Talangaï, Massina, and Linzolo, districts or villages where malaria transmission intensity is between 20 and 250 infective bites per person per year and malaria prevalence in schoolchildren is between 66% and 81%. These findings indicate that ahaptoglobinemia prevalence is correlated with the level of malaria endemicity and provide additional support for the hypothesis that malaria is the main cause of ahaptoglobinemia in African populations. The haptoglobin system may be of considerable interest in the investigation of the mechanisms of anemia in malaria.

haptoglobins; malaria

Ahaptoglobinemia is a common finding in persons living in tropical Africa but is rarely seen elsewhere (1). In a recent study of Congolese schoolchildren, we observed that ahaptoglobinemia was suppressed within a few weeks by antimalarial chemoprophylaxis and that it reappeared at its original incidence levels after interruption of chemoprophylaxis (2). The existence of a relation between malaria and ahaptoglobinemia had previously been suggested by Rougemont et al. (3) and demonstrated by Boreham et al. (4). The mechanisms involved have yet to be clearly determined (2).

The demonstration of the role played by malaria suggests that in Africa there exists a close relation between malaria endemicity and ahaptoglobinemia prevalence. To verify this hypothesis, a study was undertaken of plasma haptoglobin levels in specimens collected in various populations of the Brazzaville region of the Republic of the Congo (5, 6). These populations are subject to very different endemic levels as a result of the considerable impact of urbanization on vectorial density (7).

MATERIALS AND METHODS

Populations studied

The surveys involved four districts of Brazzaville (Massina, Talangaï, Poto-Poto,

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and Moungali) and two villages (Djoumouna and Linzolo) situated at 15 and 25 km, respectively, to the southwest of the town.

The earlier investigations describing the relation between malaria and ahaptoglobinemia were carried out in the village of Linzolo (2). We present here the overall results of five surveys of schoolchildren, and two surveys of adults and preschool-children, between November 1980 and January 1984.

The surveys undertaken in Djoumouna and in four districts of Brazzaville all took place in May 1985. They each included two samples of schoolchildren aged 6–7 and 14–15 years selected at random (in the case of Brazzaville) or representing the whole of the age group considered (in the case of Djoumouna) among children living since birth in the village or district studied.

Determination of haptoglobin levels

A sample of blood was taken by finger-prick using heparinized microhematocrit tubes. After separation, the plasma was stored at 4 C (one week) until it was tested for haptoglobin. The haptoglobin level was measured by immunonephelometry using a Technicon Autoanalyzer (Technicon Instruments, Tarrytown, NY). This method can detect haptoglobin levels as low as 5 mg/100 ml (A. Fribourg-Blanc, unpublished data).

Malaria surveys

The level of exposure to malaria was determined by means of night collections of mosquitoes on human bait and indoor-resting collections. Details of methods used and the results obtained have been published elsewhere (5, 6). The number of infective bites to which the subjects in this study are exposed is about 1,000 per year in Djoumouna, 250 per year in Linzolo, 100 per year in Massina, 20 per year in Talangaï, and less than one per year in Poto-Poto. In Moungali, the random selection of schoolchildren included only those living in the southern part of this district, where the level of exposure to malaria is about one infective bite per person per year.

The parasite rates and density were established using the method described by Trape (8); this includes the systematic examination of 200 immersion fields of the thick film (about 0.5 μl of blood examined) and determination of the parasite/leucocyte ratio on a scale of 5 classes (limits: 50, 500, 5,000, and 50,000 parasites per μl, on the basis of 8,000 leucocytes per μl). In regard to Poto-Poto, Djoumouna and Linzolo, the thick films and the blood samples for determination of haptoglobin levels were collected during the same survey. For Massina, Talangaï, and Moungali, the thick films were made during previous surveys in February and May 1984.

RESULTS

Compared prevalence of ahaptoglobinemia in six populations of schoolchildren

Table 1 indicates haptoglobin findings in schoolchildren aged 6–7 years and 14–15 years from Poto-Poto, Moungali, Talangaï, Massina, Linzolo, and Djoumouna. Table 2 indicates parasite density findings in these same schoolchildren in Poto-Poto, Linzolo, and Djoumouna. In Massina, Talangaï, and Moungali, malaria prevalence in schoolchildren aged 5–14 years was 80.9 per cent, 65.8 per cent, and 9.4 per cent, respectively, in a survey carried out the previous year.

It can be observed that a close relation exists between malariometric parameters and ahaptoglobinemia prevalence. In Poto-Poto and Moungali, where malaria endemicity is lowest, ahaptoglobinemia prevalence is also very low: less than 3 per cent on average in schoolchildren. In Djoumouna, where the level of malaria endemicity is highest, the greatest proportion of schoolchildren with no detectable haptoglobin (48 per cent) can be seen. Intermediate values are observed in Talangaï and Linzolo. In the case of Massina, ahaptoglobinemia prevalence is lower than expected in schoolchildren aged 14–15 years.

These results were analyzed according to the Kendall rank correlation test (9). A

TABLE 1

Haptoglobin findings in schoolchildren aged 6–7 years and 14–15 years in four districts of Brazzaville and the villages of Linzolo and Djoumouna, Republic of the Congo, 1980–1985

Locality or district	Haptoglobin level*																
	6–7 years							14–15 years							Total				
	0	<77 mg/100 ml	77–154 mg/100 ml	>154 mg/100 ml	Mean	SD†	No.	0	<77 mg/100 ml	77–154 mg/100 ml	>154 mg/100 ml	Mean	SD	No.	0	<77 mg/100 ml	77–154 mg/100 mg	>154 mg/100 ml	No.
Poto-Poto	1 1.8%	6 11.1%	21 38.9%	26 48.2%	167.2	88.9	54	2 4.0%	10 20.0%	21 42.0%	17 34.0%	149.8	101.7	50	3 2.9%	16 15.4%	42 40.4%	43 41.3%	104
Moungali	1 1.7%	9 15.8%	20 35.1%	27 47.4%	184.5	119.7	57	1 3.0%	6 18.2%	11 33.3%	15 45.7%	184.0	137.6	33	2 2.2%	15 16.7%	31 34.4%	42 46.7%	90
Talangaï	11 18.6%	13 22.0%	27 45.8%	8 13.6%	92.3	81.0	59	9 15.5%	21 36.2%	15 25.9%	13 22.4%	99.2	119.9	58	20 17.1%	34 29.1%	42 35.9%	21 17.9%	117
Massina	8 16.0%	14 28.0%	13 26.0%	15 30.0%	122.5	118.0	50	3 6.1%	20 40.8%	18 36.8%	8 16.3%	94.9	87.0	49	11 11.1%	34 34.4%	31 31.3%	23 23.2%	99
Linzolo	38 19.4%	71 36.2%	70 35.7%	17 8.7%	70.0	60.5	196	26 33.3%	28 35.9%	21 26.9%	3 3.9%	49.6	50.2	78	64 23.4%	99 36.1%	91 33.2%	20 7.3%	274
Djoumouna	16 55.2%	8 27.6%	1 3.4%	4 13.8%	39.3	68.8	29	8 38.1%	10 47.6%	0 0.0%	3 14.3%	52.1	95.9	21	24 48.0%	18 36.0%	1 2.0%	7 14.0%	50

* Average level of haptoglobin in a young adult French population as a reference: 110 mg/100 ml (77–154 mg/100 ml) (A. Fribourg-Blanc, unpublished data).
† SD, standard deviation.

TABLE 2

Distribution of schoolchildren by classes of parasite density, Poto-Poto, Linzolo and Djoumouna, Republic of the Congo, 1980–1985

Locality	Parasite density (classes)													
	6–7 years							14–15 years						
	0	1	2	3	4	5	No.	0	1	2	3	4	5	No.
Poto-Poto	54 100%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	54	46 92.0%	0 0.0%	0 0.0%	3 6.0%	1 2.0%	0 0.0%	50
Linzolo	42 21.4%	37 18.9%	27 13.8%	48 24.5%	36 18.4%	6 3.0	196	17 21.8%	15 19.2%	22 28.2%	19 24.4%	4 5.1%	1 0.0%	78
Djoumouna	2 6.9%	3 10.3%	6 20.7%	12 41.4%	6 20.7%	0 0.0%	29	1 4.8%	1 4.8%	6 28.6%	9 42.8%	4 19.0%	0 0.0%	21

significant correlation ($p < 0.05$) is observed for each age group between ahaptoglobinemia prevalence and malaria transmission intensity.

Variations according to age

Table 3 shows the results of a study of variations in haptoglobin levels and ahaptoglobinemia prevalence in Linzolo according to age. It can be seen that ahaptoglobinemia prevalence increases progressively with age during childhood, reaching a maximum of 32 per cent in the 10–14-year age group. It then decreases progressively, reaching 13 per cent in adults over age 40. The prevalence of low levels of haptoglobin is at its maximum in the 5–9-year age group and decreases only slightly thereafter.

Variations according to parasite density

The results of the study of the relation between ahaptoglobinemia prevalence and parasite density were presented in a previous article in regard to Linzolo schoolchildren (2). These results showed a moderate but significant increase in ahaptoglobinemia prevalence with increase in parasite density.

In Djoumouna, a positive relation was found between ahaptoglobinemia and parasite density. Of 19 schoolchildren with a class 0, 1, or 2 parasite density, only five were ahaptoglobinemic (26.3 per cent). Of 31 schoolchildren with class 3 or 4 parasite density, 18 were ahaptoglobinemic (58.1 per cent).

In Poto-Poto, none of the four children with positive thick blood films were ahaptoglobinemic: two of the children had normal haptoglobin levels, and the two others had low haptoglobin levels.

DISCUSSION

Numerous studies have reported ahaptoglobinemia prevalence in various African populations. This prevalence is low, less than 5 per cent, in Bushmen, Hottentot and South African Bantu populations (10, 11). It is also very low, less than 1 per cent,

TABLE 3

Haptoglobin levels according to age in Linzolo, Republic of the Congo, 1980–1984

Age group (years)	Haptoglobin level						
	0	<77 mg/100 ml	77–154 mg/100 ml	>154 mg/100 ml	Mean	SD	No.
<1	3 11.1%	6 22.2%	12 44.5%	6 22.2%	109.6	64.3	27
1–4	19 17.6%	29 26.8%	41 38.0%	19 17.6%	91.5	76.3	108
5–9	118 26.5%	166 37.2%	125 28.0%	37 8.3%	61.7	64.0	446
10–14	140 32.0%	159 36.4%	115 26.3%	23 5.3%	53.4	57.7	437
15–19	35 24.0%	45 30.8%	55 37.7%	11 7.5%	70.8	64.9	146
20–39	12 17.4%	23 33.3%	23 33.3%	11 16.0%	82.2	65.2	69
≥40	23 13.5%	45 26.3%	63 36.8%	40 23.4%	104.8	77.5	171
Total	350	473	434	147			1,404

in Berber Arab populations of Sahara and North Africa (12). In contrast, ahaptoglobinemia prevalence is high in tropical Africa, especially in children and young adults; values between 20 per cent and 50 per cent were observed in Nigeria (13–15), Liberia (16), Mali (12, 17), Senegal (12, 18), Gambia (4), Central African Republic (19, 20), Zaire (11), Ouganda (21), and Tanzania (21).

However, several exceptions have been reported in tropical Africa. Ahaptoglobinemia prevalence is only 4 per cent in Dakar (22), 3.2 per cent in Kinshasa (11), and 6.4 per cent in Kisangani (11), much lower values than those observed by the same investigators in rural areas of Senegal (18) and Zaire (11). Apart from the towns, low prevalences have sometimes been reported; these were generally in populations residing in high altitude regions or plateaux where ground water is rare. Thus ahaptoglobinemia prevalence is less than 5 per cent in various populations of Burundi (23, 24), and it is 10 per cent in the Masai in East Africa (21).

For a long time after the report by Allison et al. (13) in 1958 it was thought that a genetic factor could explain the high prevalence of ahaptoglobinemia in Africans. This was supported by studies of American blacks, which showed a prevalence of about 4 per cent (25–27) compared with less than 1 per cent in whites (1). Various genetic models were then suggested to explain this phenomenon (26–30), but family studies showed they could not be applied to African populations (14, 20).

Our results show that considerable differences in ahaptoglobinemia prevalence can exist between populations which are geographically close, especially in urban regions. They also confirm the observations by Giblett et al. (11), who demonstrated that the differences observed in Zaire could not be explained by ethnic factors: although the populations of Moungali, Massina, Linzolo, and Djoumouna belong to the same ethnic group (80–100 per cent are Kongo), ahaptoglobinemia prevalence varies from 2 to 48 per cent in schoolchildren.

Above all, our observations show that ahaptoglobinemia prevalence is closely related to the degree of malaria endemicity to which the population is exposed. Thus these results confirm those of our previous

study, which pointed to the possibility of suppressing ahaptoglobinemia in an African population by prolonged antimalarial chemoprophylaxis and demonstrated that potential causes of ahaptoglobinemia other than malaria, whether genetic or acquired, had a low incidence in the population studied (2).

Variations of ahaptoglobinemia prevalence in Linzolo with age are relatively slight, in accordance with the observations by Boreham et al. (4) in Gambia. These authors observed that in Keneba the ahaptoglobinemia prevalence is at its maximum between the ages of five and 19 years when it is between 29.1 and 32 per cent, and that it is 20.8 per cent in adults over 40 years old.

Similar observations were made as long ago as 1960 in Nigeria by Barnicot et al. (14), and it is mainly for this reason that malaria, alluded to when ahaptoglobinemia was discovered in Africans, was not later held responsible. Indeed, if one considers only the intravascular hemolysis due to the destruction of parasitized red blood cells, it is difficult to explain why ahaptoglobinemia prevalence is not at its maximum in children under 5 years of age with very high parasite densities, and does not fall considerably in adults with very low parasite densities in regions of high malaria endemicity. In fact, hemolysis in malaria is complex, with immune and hypersplenic factors in addition to direct destruction of parasitized red cells (34, 35). Our findings are consistent with the hypothesis that an important part of the hemolytic component of the anemia of malaria in subjects continuously exposed to the infection results from immune destruction of nonparasitized red cells. Furthermore, it is interesting to note that studies of pregnant women in tropical Africa revealed a prevalence of ahaptoglobinemia appreciably higher in these subjects than in nonpregnant women in the corresponding age group (11, 16, 31). It is generally acknowledged that there is an increase in the prevalence and severity of malaria in women during pregnancy (33). The relation between malaria and pregnancy, not yet fully understood, has been reviewed by McGregor et al (32) and Brabin (33).

The sensitivity and the specificity of the method used are crucial in the measurement of low haptoglobin values and the diagnosis of ahaptoglobinemia, which may limit the significance of the comparison of results obtained by investigators using different methods. This is particularly the case for old studies, before the introduction of immunochemical methods. However, our results suggest that it is possible to use ahaptoglobinemia prevalence as an index of malaria endemicity and that the determination of haptoglobin levels can be useful in the investigation of host/parasite relations in malaria.

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