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HEMOGLOBIN A_2 LEVELS IN MALARIA PATIENTS

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Abstract. The influence of malaria on the hemoglobin A_2 (Hb A_2) level in humans was studied in a series of 94 imported cases in Belgium. Sixty-nine of the patients were natives of Western European countries; their results are reported separately since their origin and the results of their hematological examination made it unlikely that they carried the β-thalassemia trait. The Hb A_2 level of the 94 malaria patients (mean 2.76%; S.D. 0.51%) was not statistically different from that found in 60 healthy controls (mean 2.70%; S.D. 0.38%; $P > 40$). Likewise the level of the 65 Western European patients was not statistically different from that of the same controls (mean 2.81%; S.D. 0.42%; $P > 0.10$). There was also no significant difference between the level in patients infected with a particular species of *Plasmodium* and that of the controls. No correlation was found between the Hb A_2 level and the intensity of the parasitemia or the concentration of total hemoglobin in the blood. These results are discussed in comparison with the divergent ones obtained by others and it is suggested that malaria has no significant influence on the results of surveys for the prevalence of β-thalassemia in regions of malaria endemicity.

Hemoglobin A_2 (Hb A_2) constitutes approximately 2.5% of total hemoglobin in healthy adults. Its proportion is remarkably constant among normal people of various ethnic origins, illustrating the precise quantitative control of hemoglobin synthesis.

As shown for the first time in 1957,[1] Hb A_2 is increased in most cases of thalassemia minor, often reaching double the normal concentration or more. Since that time this augmentation has frequently been used as a criterion for the diagnosis of β-thalassemia in surveys to determine the prevalence of this trait in human populations. Later, some environmental pathogenic factors were shown to also modify the Hb A_2 level, and since 1967 several studies have been devoted to the possible influence of falciparum or vivax malaria on the level of this hemoglobin. These studies have produced divergent results.

Since many surveys for the prevalence of β-thalassemia have been made in malarious areas, it is important to elucidate whether malaria can modify Hb A_2 levels and, if so, to what extent and in what proportion of the patients. To contribute to the solution of this problem, we report the results of estimations of Hb A_2 in 94 adult subjects. The determinations were made by the starch-block electrophoretic technique, which is recognized as a reference method.

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MATERIALS AND METHODS

Ninety-four adult patients suffering from untreated malaria imported into Belgium (mainly from African countries) were examined. They were hospitalized at the Leopold II Hospital of the Institute of Tropical Medicine in Antwerp, or had sought medical advice at the outpatient division of the Hospital or other medical institutions, and examination of their blood in the Laboratory of Tropical Hematology of the Institute showed the presence of malaria parasites. Of the 94 patients, 69 were natives of West European countries; their results are reported separately because their origin and the results of their hematological examination made it unlikely they could carry the β-thalassemia trait together with malaria.

The healthy controls, 60 in number, were all adult Belgians with no hematological abnormality.

Blood on dried ethylenediaminetetracetic acid dipotassium salt, and thick and thin blood films, were obtained from each patient. Routine hematological examinations were carried out according to standard methods. Malaria parasites were looked for in the thick films stained with Giemsa and the species of *Plasmodium* was determined separately by two experienced observers on the thin smears stained with May-Grünwald Giemsa. The parasites were counted in thick smears and the results were expressed as the mean number of parasites per microscopic field at 1,000×.

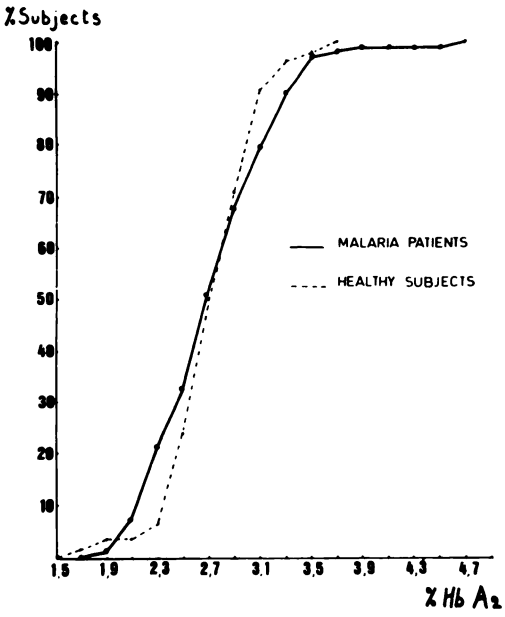

FIGURE 1. Cumulative relative frequency distributions of hemoglobin A_2 levels in subjects with and without malaria. There was no significant difference in the means of the two distributions.

The hemoglobin was examined by the sickling test, by starch-gel electrophoresis at pH 8.4, and by the alcalidenaturation test of Betke et al.[2] A few subjects with hemoglobin S were excluded from the study.

The Hb A_2 level was measured in red cell hemolysates by spectrophotometric estimation of the hemoglobin fractions separated by starch-block electrophoresis and then eluted from the starch, and expressed as the percentage of total hemoglobin. The technique used gives highly reproducible results.[3]

RESULTS

The Hb A_2 levels of the 94 malaria patients ranged from 1.9–4.6% with a mean of 2.76% (S.D. 0.51%); those of the 60 healthy controls ranged from 1.8–3.4% with a mean of 2.7% (S.D. 0.38%) and a 95% range (mean ± 2 S.D.) of 1.95–3.45%. In the 69 native West European malaria patients the mean Hb A_2 level was 2.81% (S.D. 0.47%) with a range from 2.0–4.6%; five of these patients had a Hb A_2 level which exceeded the upper limit of the 95% range of the control series, i.e., 3.45%—their levels were, respectively, 3.5%, 3.5 %, 3.7%, 3.8%, and 4.6%.

The difference between the mean Hb A_2 levels of the 94 patients and of the 60 healthy controls was not significant ($t = 0.7822, P > 0.40$) (Table 1). Likewise, the difference between the Western European patients and the control group was also not significant ($t = 1.3646, P > 0.10$) (Fig. 1).

Of the 94 patients, 69 were infected with *Plasmodium falciparum*, 18 with *P. vivax*, 5 with *P. malariae*, and 2 with *P. ovale*. The levels of Hb A_2 classified according to the species of *Plasmodium* also showed no significant differences in comparison with those of controls (Table 1).

Thirty-seven malaria patients had less than one parasite per microscopic field in their thick films; their mean level of Hb A_2 was 2.78%. Fifty-seven patients had between 1 and 400 parasites per microscopic field; their mean level was 2.75%. The difference between the distribution of the levels was not significant ($t = 0.2449, P > 0.80$). Furthermore, no correlation was found between the number of parasites and Hb A_2 levels in 45 patients with untreated falciparum malaria ($r = -0.1292, P = 0.40$). Thus, no significant difference in the Hb A_2 levels in relation to the intensity of the parasitemia at admission was found.

The possibility that the anemia of malaria might influence the Hb A_2 level was also considered. Males having less than 14 g Hb/dl blood and females with less than 12 g Hb/dl were classified as anemic. No significant difference was found between the Hb A_2 levels of the Western European patients with or without anemia (23 with, mean Hb A_2 2.93%; 19 without, mean Hb A_2 2.68%; $t = 1.5757, P > 0.10$). Furthermore, there was no significant correlation between the blood hemoglobin levels and the Hb A_2 percentages in 51 male patients ($r = 0.1719, P > 0.10$).

DISCUSSION

In addition to heterozygous β-thalassemia, several environmental pathogenic factors have been shown to modify the Hb A_2 level. Iron deficiency decreases it,[4-11] whereas it is increased in some cases of megaloblastic anemia due to folate or vitamin B_{12} deficiency.[4, 11-14] Hb A_2 has also been found to be elevated in some cases of myelofibrosis,[15] viral hepatitis,[16] and schistoso-

TABLE 1

Comparison of Hb A_2 levels in malaria patients according to Plasmodium *species with those of healthy controls*

Group	No. persons	Mean Hb A_2 (%)	S.D. (%)	Statistical significance
Healthy controls	60	2.70	0.38	
P. falciparum	69	2.78	0.47	$t = 1.0492$, $P > 0.30$, N.S.*
P. vivax	18	2.68	0.43	$t = 0.1622$, $P > 0.80$, N.S.
P. malariae	5	2.72		$t = 0.1144$, $P > 0.90$, N.S.

* N.S., values not significantly different from those of controls.

miasis,[17] and in recipients of fetal hemapoietic tissue.[18] Malaria has also been investigated, and since 1967 there have been several studies devoted to this subject. As shown in Table 2, in four of them it was concluded that malaria can sufficiently increase the level of Hb A_2 in enough subjects to induce significant differences from the control series.[19-22] This was especially the case with benign tertian malaria due to *P. vivax*. Lie-Injo et al.[20] concluded from their data that in regions of malaria endemicity the Hb A_2 estimation is not a reliable test for the diagnosis of β-thalassemia in prevalence surveys. These results led to investigations on the same subject by other workers. Three of them reached contrary conclusions—no significant difference was found;[23-25] furthermore, in a survey in Thailand a decrease of Hb A_2 was found in a series of 61 patients with falciparum malaria.[26] On the other hand, an experimental study on splenectomized *Aotus* monkeys showed no influence of falciparum malaria on the Hb A_2 level.[23]

Some of the investigations which showed that malaria had an influence on the Hb A_2 level were carried out on samples of populations in which thalassemia and iron-deficiency are prevalent; these conditions may have had unequal influences upon the Hb A_2 levels of the malaria patients and of the controls. This certainly cannot be the case in our Western European patients and controls owing to the rarity of β-thalassemia in persons of this origin and also because the results of their hematological examination did not favor a diagnosis of thalassemia or of iron-deficiency anemia. The Hb A_2 levels of these 69 European patients, as well as those of all 94 patients with imported malaria examined, indicate that malaria does not significantly modify the level of this hemoglobin, since comparison with the series of 60 controls showed no statistically significant difference. This was also the case when persons infected with *P. falciparum*, *P. vivax*, or *P. malariae* were considered separately (Table 1). Our results are therefore in accordance with those of three of the papers mentioned above.[23-25] Furthermore, no correlation was found between Hb A_2 levels and

TABLE 2

Hb A_2 levels found in malaria patients by various workers

Ref.	Place	Method*	Parasite†	No. persons	Mean (%)	S.D. (%)	Range	Comparison with controls‡
19	Venezuela	SBE	Pv	8	3.41	–	3.0 –4.0	$P < 0.001$, Incr.
26	Thailand	DSC	Pf	61	2.43	0.37	1.63–3.17	$P < 0.005$, Decr.
23	Africa§	CAE	Pf	11	–	–	2.3 –3.0	ND
23	Nigeria	CAE	Pf	30	–	–	–	ND
20	Malaysia	CAE	Pf + Pv	42	3.32	0.69	1.9 –5.1	$P < 0.02$, Incr.
21	Nigeria	CAE	*Plasmodium*	19	4.11	0.31	1.6 –7.3	$P < 0.05$, Incr.
24	Nigeria	CAE	Pf	81	2.39	0.49	1.50–3.56	$P > 0.20$, ND
22	Venezuela	CAE	Pv	8	4.60	0.46	3.9 –5.3	Incr.
25	Liberia	CAE	Pf + Pm	169	–	–	–	$P > 0.30$, ND
Present study‖		SBE	Pf + Pv + Pm	94	2.76	0.51	1.9 –4.6	$P > 0.40$, ND

* SBE, starch-block electrophoresis; DSC, DEAE-Sephadex chromatography; CAE, cellulose acetate electrophoresis.
† Pv, *P. vivax*; Pf, *P. falciparum*; Pm, *P. malariae*.
‡ Incr., increase; Decr., decrease; ND, no difference.
§ Values from European patients.
‖ Cases imported into Belgium, mainly from Africa.

the degree of anemia or of parasitemia before treatment.

Obviously, the fact that no significant difference was found between the distribution of Hb A_2 levels in large samples of malaria patients and of healthy controls does not exclude the possibility that in rare individual cases some modification of the Hb A_2 level may occur. Our results provide no conclusive evidence on this point—5 of the 94 malaria patients had a Hb A_2 level which slightly or moderately exceeded the upper limit of the 95% range of the controls, but such cases can be expected in the distribution of Hb A_2 levels in a normal population. The cumulative frequency curves in Figure 1 correspond to Gaussian distributions; thus, a few cases with Hb A_2 levels exceeding 3.45% would normally be expected. It is therefore not possible to be certain whether these levels, or some of them, were due to malaria rather than to chance. Moreover the five subjects were of Western European origin and had no signs of thalassemia or of megaloblastic anemia.

However that may be, the present investigation on cases of malaria imported into a country not affected by the disease, and in the autochthonous population in which β-thalassemia is very rare, suggests that malaria has no significant influence on the mean level and distribution of the values of Hb A_2 and, therefore, the presence of malaria does not influence the validity of the estimations of this hemoglobin used in detection of carriers of β-thalassemia genes in population surveys. Nevertheless, the results of such surveys may be altered by the numerous cases of iron-deficiency anemia which are observed in many regions where malaria is endemic. Since iron-deficiency anemia often decreases the Hb A_2 level in thalassemic, as well as in non-thalassemic individuals, the results of many surveys based on Hb A_2 levels may only be considered as minimal estimations.

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