MALARIA MORBIDITY REDUCING IN A POPULATION OF HEMOGLOBIN E CARRIERS

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Abstract
The ability of protection of Hemoglobin E (HbE) against malaria caused by Plasmodium falciparum is still in debate. We have recognized the existence of an abnormal Hb in a severe pandemic area of malaria. All malarial patients hospitalized at the health Post of Thanh Commune in the epidemic season of 2003 were included in the study. For the control group (n=157), the frequency of HbE gene is 47% including 22% of EE and 51% of AE gene. These proportions are similar to those reported in general population of Van Kieu ethnic minority (43% and 50% respectively). For the malarial patients group (n=45), the frequency of HbE gene was 31% including 9% of EE and 44% of AE. Both control and patients groups were similar in the aspects of age and sex. The frequency of AA gene carrier was higher significantly in the patient group ($p<0.03$). These results show that the presence of HbE genotype may have a protection role against malaria.

1. INTRODUCTION

Health care for ethnic minorities is an important policy of the State of Vietnam as this is a residential population in remote areas with very difficult life. The Bru Van Kieu in Huong Hoa district, Quang Tri province is an ethnic group living in the harsh natural conditions and has a regular exposure to a "traditional" disease of the mountains in the central Vietnam, it is malaria. Two questions are posed, those are: Why malaria is hard to eradicate here after so many efforts of the Vietnamese government which consumed a big amount of manpower and material resources? And why Van Kieu ethnic people here still exist hundreds of years without perishing by this pandemic disease? Those are the big questions that those who study ethnic Van Kieu and malaria also wish to ask.

To help find answers, our research group at Hue Medical University since 1994 has conducted many studies on epidemiology, immunology of malaria on the Bru Van Kieu ethnic group in Thanh Commune, Huong Hoa district, Quang Tri province. With the research results obtained we found that the characteristics of habitat, the customs, the economic situation and nutrition of people in this place are important factors which have been contributing to the status of long-term survival and sustainability of malaria here.

But how people in Huong Hoa Van Kieu still exist and the disease has not been able to wipe them out from years? We can immediately find that the answer is at least partly related to genetic characteristics of natural selection. From the late 1940s, when studying the relationship between sickle cell anemia, containing HBF, JBS Haldane argued that: "Red blood cells of anemic heterozygotes smaller than normal are better resistance with hypotonic solution. So, at least it has been

thought that they (red blood cells) would be better resistance against the penetration of malaria parasites "[9]. Chotivanich [2] and Win [14] also demonstrated that the presence of hemoglobin E (HbE) in erythrocytes may be a factor in red blood cells to help naturally resistant to the penetration of the parasite and therefore prevent malaria infection.

Besides the above publication, the study of Southeast Asia has also noted that there are many ethnic groups in this area carrying a special type of hemoglobin, other than the usual type of hemoglobin is HbAA. The study's authors in Thailand, Pravatmuang [10] Tanphaichitr [11], have also given similar results. Especially, Hutagalung [6] has noted that the treatment with artemether derivatives in patients with Hb E RBC would have been much more favorable (time of healingwas faster and time for eradication of blood parasites in HbE patients was shorter than those in HbA ones).

To study if Bru Van Kieu ethnic people in Huong Hoa district, Quang Tri Hb having structure naturally favorable for anti-malaria, we have associated with the University of Mediterranee in Marseille, France, to conduct a research on Hemoglobin types of Bru Van Kieu people in order to clarify the hypotheses about the natural resistance mechanism of red blood cells against malaria parasites.

2. SUBJECTS AND METHODS

2.1. Study subjects

The study was performed in Thanh Commune, Huong Hoa district, Quang Tri province. The entire population consists of 9 villages. The population of the commune was 2203 people. All are of Bru Van Kieu ethnic minority. These areas are classified as the most severe pandemic malaria ones. Study subjects included 2 groups: control group and malaria group.

All 157 volunteers served as controls. They belonged to 35 families. Each of them will be investigated on the name, age, gender and family relations.

The malaria group included those coming to commune health centers with symptoms of clinical malaria and the malaria positive parasite tests found. For those who were positive for *P. falciparum*, their blood samples were carried out before treating with artemether and were followed up until recovery. Blood samples were immediately centrifuged and red cells stored at -20 ° C until analysis. There were 45 patients in this group.

2.2. Methods

2.2.1. Malaria parasite test

Malaria parasites were identified by the microscope method. Each patient was being done two tests, one with a drop of thick smear and one with drop of thin one. Smears were read under an objective lens 100. All positive patients were confirmed by PCR after that.

2.2.2. Hemoglobin (Hb) type determination Hb type was determined by the method of electrophoresis (Sebia) on semi-automatic system "Hydrasys" [13] (as directed by the manufacturer). The first step, we performed gel electrophoresis on alkalin. Based on a control sample containing Hb A, this step allowed to determine Hb A and Hb non-A. The samples with Hb non-A were then run on acid gel electrophoresis which allowed to determine precisely other types of Hb (E or C).

3. RESULTS

In the control group of 157 volunteers, the sex ratio (male/female) was 1.07; the age range was 4 to 70; under 21 year-old people accounted for 58% (Table 1). The rates of Hb types were: Hb AA 27%, Hb AE 51% and Hb EE 22% (Table 1).

Table 1. Percentage of people in the control group at different ages carrying different types of Hb found in Thanh Commune

Age	4-10		11-15		16-20		21-40		>41		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
AA	14	33	8	19	4	9	7	16	10	23	43	27
AE	25	31	11	14	11	14	14	18	19	24	80	51
EE	8	24	6	18	4	12	10	29	6	18	34	22
Total	47	30	25	16	19	12	31	20	35	22	157	100

The malaria group included all 45 patients expressing symptoms of clinical malaria and positive for P. falciparum, 30 male and 15 female, aged 4 to 45 years old. The rate of Hb types was: 47% of AA, 44% of AE and 9% of EE (Table 2).

Table 2. Percentage of people in the malaria group at different ages carrying different types of Hb found in Thanh Commune

Age	4-10		11-15		16-20		21-40		>41		Total	
	n	%	n	%	n	%	n	%	n	%	n	%
AA	9	43	4	19	6	29	1	5	1	5	21	47
AE	6	30	2	10	5	25	5	25	2	10	20	44
EE	2	50	0	0	1	25	5	25	0	0	4	9
Total	17	38	6	13	12	27	11	16	3	7	45	100

Compare the rates of Hb types between the cases and the controls by $\chi 2$ test, we found that the differences were statistically significant ($\chi 2 = 7.433$, $p < 0.03$). Hb AA ratio in the group of malaria was very high: 47% versus 27% in the control group. In contrast, the rate of Hb EE in cases decreased 9% compared with 22% in the control group. HbE common rate was 53%.

Most malaria cases were in young people. In our study, nearly 80% of cases of malaria were under 21. Therefore, we compared Hb type proportions between the control group and the malaria group of people under 21 (Table 3).

Table 3. Differences in the types of Hb ratio in the age group of <21

Hb types / Group	AA					
	n	%	n	%	n	%
Control	26	28.57	47	51.65	18	19.78
Malaria	19	54.29	13	37.14	3	8.57

Hb ratio of two groups was very different. In comparison with the control group, the Hb AA ratio in the malaria group was very high: 54% versus 29% of the control group. The rate of Hb EE and AE in malaria group was lower: 9% of Hb EE compared with 20% of the control group, and 37% of Hb AE compared with 52% of the control group. The difference in the ratios of Hb types between these groups was statistically significant ($\chi 2 = 7.702$, $p < 0.02$).

4. DISCUSSION

The previous epidemiological data have shown that some genetic variations of red blood cells resulting from natural selection were aimed at the purposes of fighting against the susceptibility of malaria. This was particularly true to some ethnic minorities scattered in different geographical areas of Southeast Asia. For Bru Van Kieu ethnic groups living in Thailand, the proportion of people with HbE was 50% [4], in Laos was 43% [3]. Our study found this was 53%, this was also consistent with the data in neighboring countries (although slightly higher), and was a representative number for this ethnic group in the region.

For patients under age 21, the further difference between the rates of HbE (71.43% in controls versus 45.71% in patients) and HbAA (28.57% in controls and 54.29% in patients) carriers with $p < 0.02$ showed the clearer role of Hb types in the morbidity of the disease. In another way, this again confirmed the role of HbE in reducing the incidence of malaria, especially in young people.

The data of our research showed quite clearly that individuals carrying HbE either homozygous or heterozygous have malaria infection rates low. In this work, we could not distinguish between the case of complications and no complications because patients' number was limited. In the future study, we will try to distinguish the two cases to see if there is any difference between the two groups.

So far, the mechanism of this resistance has not been completely clarified. In *in vitro* experiments it was noted that the interference and growth of the parasite declined clearly HbE erythrocytes [12],[15]. Furthermore, when macrophages (i.e. monocytes of normal) were incubated with HbE erythrocytes from individuals infected with malaria parasites, researchers also found that the abnormal membrane of HbE red cells have helped the majority of red cells resistant to the penetration of the parasites [2],[1]. However there were some clinical studies in Burma and Thailand that did not see the protection of HbE red blood against malaria parasite [7],[8]. In contrast, other studies, also in Thailand and Myanmar have noticed that HbE red blood cells were capable to protect against cases of severe malaria [5], although the concentration of parasites were not altered. Some authors also analyzed after further study that the resistance to illness was done through their resistance to the increase of parasites in the blood [2]. In our study, the concentration of the parasite in all three groups (AA, AE, and EE) was approximately equal. This speaked to the possibility that in the Bru Van Kieu ethnic minority, HbE red blood cells were despite resistant to the disease as well as reduce the severe cases but this was not much related to the concentration of parasites in the blood.

In this study, due to the limitation of time and budget, we could not go further to study the protection mechanism of HbE red blood cells against malaria parasites. We hope in the future we could realize more studies to help explain the genetic mechanisms in the populations of Bru Van Kieu which lead to the long-term survival of this ethnic group in severe pandemic area of malaria.

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