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OBSERVATIONS ON THE RESISTANCE OF HB.E-THALASSAEMIA
DISEASE TO INDUCED INFECTION OF PLASMODIUM VIVAX

by

R. N. Ray,¹ J. B. Chatterjea² and R. N. Chaudhuri³
(School of Tropical Medicine, Calcutta, India)

INTRODUCTION

Interest in the study of a possible relationship between malaria and haemoglobinopathy was aroused by the pioneer observations of Allison (1954) who demonstrated that the majority (13 out of 15) of subjects with haemoglobin S-trait offered resistance to induced Plasmodium falciparum infection, when in the control group 14 out of 15 non-sicklers suffered from the same infection. Subsequent studies confirmed that sicklers (Hb.S-trait) in general offer a significant resistance to P. falciparum infection (Raper, 1955; Edington & Lehmann, 1956). Sicklers did not, however, show similar resistance to P. malariae (Allison, 1954; Colbourne & Edington, 1956).

Available information on the relationship between sickle cell trait and P. vivax infection is scanty and inconclusive. Boyd & Stratman-Thomas (1933) observed that the negroes in the United States of America were somewhat resistant to induced P. vivax infection as compared with the white people. The concept of haemoglobinopathy was unknown at that time and no attempt was made to link up this resistance with Hb.S-trait.

¹ Reader in Haematology, School of Tropical Medicine, Calcutta

² Professor of Haematology, School of Tropical Medicine, Calcutta

³ Professor of Tropical Medicine and Director, School of Tropical Medicine, Calcutta

In our study on the relationship between malaria and haemoglobinopathy, it was observed that Hb.E-thalassaemia subject was resistant to induced P. vivax infection (Chatterjea et al., 1956). No such resistance was, however, demonstrable with induced P. falciparum infection (Chatterjea, 1959). Further observations in a larger series of Hb.E-thalassaemia along with few other haemoglobinopathic disorders are recorded in the present communication.

MATERIALS AND METHODS

The induction experiments with Plasmodium vivax were done on six different occasions. A total of 18 subjects were studied, consisting of Hb.E-thalassaemia - 11, homozygous thalassaemia - 1, homozygous E disease - 1, sickle cell anaemia (homozygous) - 1, sickle cell disease - 1 (Hb.S content was 80%; family study was not possible; status Hb.S-thalassaemia or sickle cell anaemia), thalassaemia trait - 2 and Hb.E-trait - 1. In the control group there were 14 subjects who were recovering from iron deficiency anaemia or were suffering from non-haemoglobinopathic disorders.

Routine clinical and standard haematological investigations were done to categorize the haemoglobinopathic status. The control cases were also studied in a similar way to exclude the possible existence of any haemoglobinopathic abnormality. The foetal Hb. was estimated according to the method of Singer et al. (1951). Erythrocytic glucose-6-phosphate dehydrogenase (G-6-PD) activity was estimated according to a method adapted from Kornberg & Horeker as modified by Marks (1957). The stability of erythrocytic reduced glutathione (GSH) was tested according to the method of Beutler (1957).

Procedure of the experiment

The donor of Plasmodium vivax was selected from benign tertian malaria patients who had already several bouts of febrile paroxysms. In the donor's blood at the time of bleeding, 1.5% to 2.0% of red cells were parasitized. The blood was collected in a sterile bottle containing acid citrate dextrose solution. Immediately after collection, the blood was injected intramuscularly both in the haemoglobinopathic subjects and in the controls. Average adults received 10 ml and the children received 5 ml of blood. Following such injection all the cases were observed regularly both clinically and haematologically for evidences of malarial infection, as long as circumstances permitted.

RESULTS

Hb.E-thalassaemia disease

The experiments were done on six different occasions employing a different donor each time. It was observed that out of 11 cases of Hb.E-thalassaemia, only two developed malaria. These two cases had typical bouts of fever and parasites were found respectively on the seventh and 36th day following injection. The remaining nine cases proved resistant to infection with P. vivax; these were followed carefully for six months to a year with periodic check-up of blood. None of them had any fever and no parasites could be demonstrated.

Other haemoglobinopathic conditions

One case of homozygous thalassaemia (age 1-1/2 years, Hb. 5.51 g %, Hb.F 85.0%, spleen 2.5 cm), one case of thalassaemia trait (age 50 years, Hb. 10.73 g%, Hb.F 7.74% with a palpable spleen - 7.5 cm), and one case of Hb.S-disease (age 20 years, Hb. 4.35 g%, Hb.S. 80%, spleen 2.5 cm) showed resistance to B.T. malaria. The other cases consisting of one case of homozygous sickle cell anaemia, one case of homozygous E disease, one case of thalassaemia trait and one case of Hb.E-trait showed no resistance. The results are briefly shown in Table 1.

Control group

Thirteen out of 14 subjects showed clinical and parasitological evidences of malaria. Parasites were demonstrable between seven and 22 days following injection of blood.

Correlation between malaria and other factors

Age of the subjects: No definite relationship could be found out between the age of the patient and resistance to malaria. Ages of Hb.E-thalassaemia showing resistance to P. vivax ranged from six years to 21 years. In the controls and two subjects of Hb.E-thalassaemia developing malaria, the age range was similar. The only case of homozygous thalassaemia who also proved resistant was only 1-1/2 years old.

TABLE 1. DATA ON THE RESULTS OF INOCULATION OF MALARIAL BLOOD

Subjects	Total No.	Malaria infection positive	Malaria infection negative
Hb.E-thalassaemia	11	2	9
Homozygous thalassaemia	1		1
Sickle cell anaemia	1	1	
Hb.S-disease	1		1
Thalassaemia trait	2	1	1
Homozygous E disease	1	1	
Hb.E-trait	1	1	
Controls	14	13	1

Anaemia in the subject: There was no significant correlation between the degree of anaemia and the susceptibility to malarial infection. Subjects of Hb.E-thalassaemia with haemoglobin level as low as 2.32 or 3.19 g% did not develop malarial infection. In the control group, both the normal and the anaemic subjects showed equal susceptibility to malarial infection. The hypochromic red cells of two iron deficiency anaemia included in the control group did not appear to offer any resistance to parasitic infection. Similarly, the two cases of Hb.E-thalassaemia developing malarial infection had grossly hypochromic red cells.

Reticulocyte count: Reticulocyte count per se was not related to the susceptibility or otherwise to malarial infection.

Splenomegaly: Size of the spleen did not appear to influence the susceptibility to malaria. One case of Hb.E-thalassaemia, splenectomized five years ago, and showing a Hb. level of only 2.32 g% at the time of experiment, did not develop malarial infection.

Level of foetal Hb.: No correlation could be established between the level of foetal haemoglobin and resistance to malaria. In the control group, one case of subacute myeloid leukaemia with foetal Hb. level of 50.5% (no abnormal Hb.) and two cases of

Hb.E-thalassaemia with foetal Hb. level of 28.5% and 50.5% did not offer any resistance to malaria. The case of homozygous thalassaemia with 85.0% foetal Hb showed resistance to malarial infection; but similar resistance was shown by the majority of E-thalassaemia subjects with Hb.F level ranging from 7.76 to 48.4%.

Presence of any particular abnormal haemoglobin: Homozygous state of thalassaemia showed resistance to malaria in only one case studied. But the homozygous state of S and of E (only one case of each) showed no resistance to malaria. One case of thalassaemia trait (aged 50 years, Hb. 10.73 g%, Hb.F 7.74% with a spleen 7.5 cm) offered resistance to malaria. But the other case of thalassaemia trait (aged 36 years, Hb. 11.5 g%, Hb.F 2% and no palpable spleen) and one case of Hb.E trait studied did not show any resistance to malaria. Double heterozygote state of thalassaemia and Hb.E genes appeared to have offered resistance in nine out of 11 cases studied.

Erythrocytic G-6-PD and GSH stability: Erythrocytic enzyme studies (Table 2) in eight cases of Hb.E-thalassaemia showing resistance to P. vivax had normal or high G-6-PD activity and stable GSH in six cases; G-6-PD activity was low with unstable GSH in two cases. In two cases of Hb.E-thalassaemia susceptible to P. vivax, G-6-PD activity was normal and GSH was unstable in only one of them. The case of homozygous thalassaemia had almost nil G-6-PD activity.

Resistant cases, each of Hb.S-disease, thalassaemia trait and one in control group did not have any significant abnormality of G-6-PD or GSH.

Differential susceptibility to P. falciparum and to P. vivax: One subject of Hb.E-thalassaemia aged 14 years (Hb. 6.09 g%, reticulocytes 18.0%, spleen 12 cm and foetal Hb. 21.0%) showed easy susceptibility to induced falciparum infection in 1957. The same case in 1960 was resistant to P. vivax.

Any therapeutic value of induced malaria: In our series, following infection the subjects did not show any clinical or haematological improvement. On the contrary, the majority became worse off after the infection.

TABLE 2. DATA ON G-6-PD ACTIVITY AND GSH STABILITY OF RED CELLS AND THEIR RELATIONSHIP WITH THE OCCURRENCE OF INDUCED MALARIA

Cases	B.T. malaria	G-6-PD		GSH	
		N or high	Low	Stable	Unstable
Hb.E-thalassaemia (8	Negative	6	2	6	2
(2	Positive	2	-	1	1
Sickle cell anaemia 1	Positive	1	-	-	1
Hb.S-disease 1	Negative	1	-	-	1
Homo-thal 1	Negative	-	1	-	1
Thal-trait 1	Negative	1	-	1	-
Controls (6	Positive	6	-	3	3
(1	Negative	-	-	1	-

DISCUSSION

Results of direct experiments designed to test for any possible resistance that may be offered by haemoglobinopathic subjects to malaria, indicated significant resistance of Hb.E-thalassaemia subject to Plasmodium vivax. In a series of 11 patients with Hb.E-thalassaemia disease, only two could be infected, while in the control series, 13 out of 14 were readily infected. Such infection could not be induced even in a splenectomized child with Hb.E-thalassaemia disease. Most of these children were greatly anaemic with low general condition and yet they had showed this resistance. Similar resistance was also seen in homozygous thalassaemia but not in a typical thalassaemia trait, haemoglobin E trait or haemoglobin E homozygote. It is further interesting to note that resistance in Hb.E-thalassaemia was to P. vivax but not to P. falciparum. The observed resistance to P. vivax could not be correlated with any of the following parameters: Hb.F content, reticulocyte count, G-6-PD activity or stability of reduced glutathione. It should, however, be mentioned that in a

similar study from Thailand, both Hb.E trait adults and Hb.E-thalassaemia children developed infection with P. vivax (Kruatrachue et al., 1961). In a survey of malarial infection in relation to the distribution of Hb.E in various age-groups of a malarious area in Thailand, subjects with Hb.A and Hb.E were found to be equally susceptible to malaria (Kruatrachue et al., 1962).

The resistance of Indian subjects to P. vivax and African subjects to P. falciparum may be reconciled by the fact that malarial parasites in general do not thrive well in haemoglobinopathic red cells. Well documented data on the resistance of Africans to P. vivax are not available. Relatively scanty data on P. falciparum infection in Indian subjects do not completely exclude the possibility of some resistance to this species, in addition to striking resistance to P. vivax.

The mechanism of resistance as shown by Hb.E-thalassaemic subjects from India is not, however, clear. There is no direct evidence to show that presence of any abnormal haemoglobin or thalassaemia gene per se has any protective role. In the present series, the observed resistance could not also be explained by the amount of Hb.F present or the associated G-6-PD deficiency. Also in a recent study from Thailand G-6-PD deficiency could not be always correlated with resistance to malarial infection (Kruatrachue, et al., 1962).

In an over-all assessment of the available evidences relating to the resistance shown to malarial infection it would appear that besides abnormal haemoglobin and G-6-PD deficiency, there must be other factors. Only a minority of American negroes have these traits and it is generally agreed that many more of them show after inoculation a considerable resistance against several species of malarial parasites, including P. vivax and P. knowlesi. The differential susceptibility of American negroes and American whites - who had no previous exposure to the disease suggests that other factors, probably under complex or polygenic control are involved (Livingstone, 1961). In this context, the differential susceptibility of Indian and Thai subjects may be due to other associated factors not directly related to haemoglobin variants.

Caminopetros (1958) observed that in a case of Mediterranean anaemia who had a coincidental malarial infection, there was improvement of anaemia. Impressed with this observation, he inoculated seven subjects of Mediterranean anaemia with malaria.

He reported that malaria had a beneficial effect on anaemia. In the present series no such beneficial effect was observed. On the contrary, following malarial infection there was deterioration of clinical and haematological conditions, as expected.

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SUMMARY

Subjects with Hb.E-thalassaemia disease were found to offer significant resistance to induced P. vivax infection. In a series of 11 patients with this disease, only two could be infected, when in the control group 13 out of 14 were readily infected. The observed resistance to P. vivax could not be correlated with Hb.F content, reticulocyte count, G-6-PD activity or stability of reduced glutathione in red cells.

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