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HAEMOGLUBINOPATHIES AND MALARIA IN THAILAND¹

by

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1. Introduction

Although it is known that Haemoglobin-E and thalassaemia traits are prevalent in South-East Asia, the selective advantages for individuals who possess these abnormal genes are still not clear. Some attempts have been made to study the protective value against P. falciparum of these genetic variants (Na-Nakorn et al. 1956, Das Gupta et al. 1956, Kruatrachue et al. 1960, 1961, Flatz et al. 1964) but the results have not been conclusive.

In certain areas of Thailand there is a surprisingly high incidence of Haemoglobin-E. For instance, in the area in which the present survey was carried out the incidence of this gene was 30-50%. The incidence of β -thalassaemia was found to be 3.2% to 10.6%. There is some evidence that the incidence of α -thalassaemia is also high (Pootrakul et al. 1966). Such high frequencies of deleterious genes are usually held to indicate selective advantages for the relevant individuals. A high prevalence of a gene lethal in the homozygous state can only be accounted for by postulating some selective advantage derived from possession of the gene in a single heterozygous state.

In the present communication, further data is presented relevant to the hypothesis of selective advantages offered by heterozygosity for Haemoglobin-E and thalassaemias in P. falciparum infections.

2. Material and method

Surveys of the incidence of haemoglobin types and of malaria were performed in three provinces in Thailand where a high malaria prevalence was anticipated: Rayong, Nakorn Rajsrima and Nakorn Nayok.

Blood samples were collected, mostly by femoral vein puncture, from 600 infants and children under three years of age. For the diagnosis of haemoglobin types the blood samples were treated as follows:

Diagnosis of Haemoglobin-E. Haemoglobin-E was identified by starch block electrophoresis (Kunkel & Wellenius 1955). Many attempts were made for the elution of the E component but the results were not reproducible and latterly the separations were made by chromatography using DEAE-Sephadex (Huisman et al. 1961). In our series, mean of HbE is 21.77 ± 8.14 (The study of 79 cases).

Diagnosis of the thalassaemia trait. The thalassaemia trait was diagnosed by the elution of the Haemoglobin A₂ component after starch block electrophoresis. In 40 normal individuals the normal values of Haemoglobin A₂ varied between 1.3% to 3.5% with high frequency between 2.5% to 2.9%. Thus up to 3.5% was regarded as within normal limits (Mean \pm 2 S.D = $2.70 \pm 1.32\%$).

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For the diagnosis of thalassaemia-like disorder (thalassaemia trait), the following procedures were carried out:

(i) Osmotic fragility test. Fragility test in serial dilutions of saline from 0.16% to 0.48% at intervals of 0.02% were made. Blood samples were studied from the following groups: 72 normal individuals and 58 with either α - or β -thalassaemia genetically proved trait. The 0.36% saline solution showed clear separation of the two groups. At this dilution in individuals with normal haemoglobin haemolysis was not less than 82%; in the thalassaemia traits it did not exceed 79%.

0.36% saline solution was therefore used for screening in field fragility tests. The percentage of haemolysis in this saline dilution were calculated by standard procedures. Individuals showing fragility of less than 79% were regarded as having decreased fragility.

(ii) Plasma iron. The syringes, test-tubes and all glasswares used in the survey were iron free and siliconized in order to minimize haemolysis. The separation of plasma was done within one to two hours after collection. Plasma iron was estimated after the method of Schade et al. (1964). The normal value in Thai people was 47-214 mgm% (Wasi et al. 1966).

Only samples with decreased osmotic fragility and normal levels of plasma iron and haemoglobin A₂ were classified as "thalassaemia-like disorder". It is possible that thalassaemia trait with normal haemoglobin A₂ levels were included in this group. Cases with decreased fragility associated with low plasma iron levels were discarded. However, cases with normal fragility, in spite of low plasma iron were included in the AA individuals.

Double heterozygosity for β -thalassaemia and the HbE gene and homozygosity for HbE were excluded. Nevertheless, in this study the so-called "individuals with E" possibly included also subjects heterozygous for HbE with one or two thalassaemia genes (Tuchinda et al. 1964).

By the criteria described above, 58 out of 600 cases were discarded, including two homozygous HbE, 12 double heterozygous for thalassaemia and HbE genes (Thalassaemia HbE disease), one suspected thalassaemia-HbH and 43 cases with decreased fragility and low plasma iron level. The remaining 542 cases were used in this study.

In most cases, foetal haemoglobin was estimated using the method of Singer et al. (1951).

Diagnosis of malaria infection. In all cases, two thick peripheral blood films and one thin film were examined. The diagnosis and the calculation of parasite densities in survey samples were done as previously described (Kruatrachue et al. 1962).

Studies of the hospital cases. Studies were also made of Plasmodium falciparum infections in infants and children under three years of age admitted to Praputhabath and Saraburi Hospitals. Samples of blood were collected and treated as above; glucose-6-phosphate dehydrogenase (G-6-PD) concentrations were determined in the erythrocytes, using the brilliant cresyl blue dye screening method.

In all cases the initial parasite counts were made by counting the number of infected cells in 1000 erythrocytes in thin film smears stained with Wright's stain.³ The red blood cell count per mm.³ was also determined and the number of parasites per mm.³ was then calculated.

3. Results

The incidence of haemoglobin types and the incidence of P. falciparum malaria are shown in Table 1.

It can be seen that the incidence of individuals with HbE ranged from 23.0% to 51.9%, with an average of 28.0%. Of the two types of "thalassaemia individuals" α -thalassaemia predominated, the incidence varying from 16.7% to 19.3% (with an average of 18.5%).

The incidence of P. falciparum infection in the areas Rayong, Nakornrajsima, Nakorn Nayok were more or less the same.

Relation of individuals with haemoglobinopathies and falciparum malaria. The samples were analysed in two ways; according to i) age groups (infants and children one to three years of age) and ii) the degree of local endemicity of malarial infection (P. vivax and P. falciparum) on the basis of whether the incidence was greater or less than 15%. The results are shown in Tables 2 and 3 respectively.

For statistical analysis the groups with α and β thalassaemia traits were pooled and compared with groups with HbE trait and with normal. As will be seen from study of Tables 2 and 3, only in the one to three years age group and in the villages where the infection rate was higher than 15% were numbers of infected individuals high enough to permit statistical analysis (that is, the expected frequency in every cell is higher than 5). In the circumstances mentioned above the χ^2 test with Yate's correction indicated that the samples were taken from a universe in which the incidence of falciparum infection would be the same.

Comparison of parasite densities. For the comparison of parasite densities between individuals with various haemoglobin types, the data obtained from the survey were inadequate because of the low parasitaemia. To obtain a relatively large population of children with high parasitaemias 82 cases of ill infants and children under one to three years of age, admitted to Saraburi and Praputhabath Hospitals were included (Table 4).

A level of more than 50 000 parasites per mm³ was chosen as the danger point in falciparum infection, since data in infants and children under three years of age infected with P. falciparum indicated that the mortality rose from 2.8% to 29.3% when this level of parasitaemias occurred. Since the significance of G-6-PD deficiency in regard to parasite densities is not yet clear (Kruatrachue et al. 1966), cases with this enzyme defect, regardless of the haemoglobin type, were rejected. There was no evidence that the proportion of the individuals with parasite counts higher than 50 000 per mm³ differed significantly among the three groups.

4. Discussion

It can be seen from Table 1 that, although the malarial endemicity of the areas studied was similar, the distributions of HbE individuals and β -thalassaemia trait in them were not uniform, the difference in frequencies being statistically significant ($P < .01$).

In terms of infection rate and parasite densities, it was not possible to demonstrate that HbE and thalassaemia traits (α and β) conferred any selective advantages for falciparum infection.

Up to the present, disadvantages of the homozygous state for HbE which is of very mild pathological importance have not been demonstrated. It is suspected that the advantages in regard to malarial infection in heterozygous persons, if present, must be very small. In such circumstances a very large number of cases is needed for analysis. However, if heterozygous E carries any advantage in falciparum infection, in a number of cases sufficient for the calculation of χ^2 test, the results should show some trend, even if the significance is not statistically demonstrated. In our data no such trend was observed either insofar as incidence or density was concerned.

It is worth noting that the incidence of thalassaemia-like disorder (α) in Thailand is alarmingly high. The present findings fit well with the high incidences of hydrops foetalis (Pootrakul et al. 1966) and high incidence of thalassaemia α found in this country in 57 families in which one or more had thalassaemia H disease (Wasi et al. 1964). The incidence of haemoglobin Bart's in the cord blood, is 6.2% (Pootrakul et al. 1966). Thus, the average of H genes or recently the so-called α th₂ genes in our sample is 12.2%. Homozygosity for β -thalassaemia gene however is associated with lethal anaemias; homozygosity of α -thalassaemia is not compatible with life (Lie-Injo et al. 1965). Selection in favour of heterozygous states in β -thalassaemia must be intense, and only suggestive geographical evidence has been adduced to implicate falciparum malaria as the selective agent (WHO 1966). However, the present data, both in respect of falciparum infection rates and parasite densities, do not indicate that α - or β -thalassaemia traits confer any selective advantages in the malarial environment.

Our results conflict with studies of frequency of E gene distribution in relation to the incidence of falciparum infections Thailand (Flatz et al. 1964). We are convinced however that the interpretation of the gene distribution depends not only on the prevailing malaria incidence, but also on the interaction of the abnormal genes among the populations concerned. The apparent correlation of malaria incidence and a given gene, may be purely coincidental or a third unknown factor may be involved which is not P. falciparum. If correlation between these abnormal gene frequencies and malarial incidence is real, it should be possible to demonstrate clearly in many malarious areas in Thailand.

The results of our studies of fatal cases of P. falciparum with parasitaemia higher than 50 000 mm³ in infants and children do not indicate any clear difference between the series of normal and abnormal haemoglobins.

Details of these studies will be provided at a later stage.

SUMMARY

Studies of Plasmodium falciparum infection rates and parasite densities among infants and children under three years of age in Thailand do not support the hypothesis that Haemoglobin-E and the thalassaemia traits confer a selective advantage in falciparum infection. However, further study of the mortality rate in relation to abnormal haemoglobin genes in this infection is needed.

RESUME

Dans certaines régions de Thaïlande, on observe des fréquences relativement élevées des thalassémies α et β et de l'hémoglobinoïse E. On considère généralement qu'à un tel niveau, ces fréquences sont révélatrices d'avantages sélectifs chez les sujets atteints. Une forte prévalence d'un gène létal à l'état homozygote ne s'explique en effet que si l'on admet que la possession du gène à l'état hétérozygote confère quelque avantage sélectif.

Ce document présente des données touchant l'hypothèse selon laquelle l'hétérozygotisme pour les gènes de l'hémoglobinoïse E et des thalassémies conférerait des avantages sélectifs en cas d'infection à Plasmodium falciparum dans trois provinces de Thaïlande où l'on s'attendait à trouver une forte prévalence du paludisme.

Or l'étude des taux d'infection à P. falciparum et des densités parasitaires parmi les nourrissons et les autres enfants de moins de trois ans en Thaïlande ne confirme pas cette hypothèse. De nouvelles recherches sur les rapports entre le taux de mortalité et la fréquence des gènes des anomalies hémoglobinoïques sont toutefois nécessaires.

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TABLE 1. INCIDENCE OF HAEMOGLOBINOPATHIES AND MALARIA IN THE PROVINCES SURVEYED

Province	Number examined	Persons with						Malaria positive		P. falciparum positive	
		HbE		β -thalassaemia		α -thalassaemia		Number	%	Number	% ^a
		Number	%	Number	%	Number	%				
Rayong	348	80	23.0	12	3.4	64	18.4	44	12.6	28	63.6 8.0
Nakorn Rajsrima	54	28	51.9	2	3.7	9	16.7	6	11.1	4	66.7 7.4
Nakorn Nayok	140	44	31.4	16	11.4	27	19.3	19	13.6	11	57.9 7.9
Total	542	152	28.0	30	5.5	100	18.5	69	12.7	43	62.3 7.9

^a The upper and lower lines indicated the % of P. falciparum infection calculated from malaria positive cases and from total cases respectively.

TABLE 2. INCIDENCE OF MALARIA IN RELATION TO HAEMOGLOBINOPATHIES IN INFANTS AND THOSE UNDER THREE YEARS OF AGE

Individual with	Infants (under 1 year)			1-3 years of age		
	Parasite positive	Parasite negative	%	Parasite positive	Parasite negative	%
HbE	1 (1)	45	(2.2)	13 (8)	93	(7.5)
Thalassaemia-like disorders	2 (0)	29	(0)	9 (4)	60	(5.8)
β -thalassaemia	0	7	(0)	5 (3)	18	(13.0)
Normal Hb	3 (0)	70	(0)	36 (27)	151	(14.4)

The figures in brackets indicate the number and percentage of individuals expected with falciparum infection.

TABLE 3. INCIDENCE OF MALARIAL INFECTION IN RELATION TO HAEMOGLOBINOPATHIES AND DEGREE OF MALARIAL ENDEMICITY

Individuals with	Malaria infections less than 15%			Malaria infections more than 15%		
	Parasite positive	Parasite negative	%	Parasite positive	Parasite negative	%
HbE	2 (1)	77	(1.3)	12 (8)	61	(11.0)
Thalassaemia-like disorders	3 (1)	57	(1.7)	8 (3)	32	(7.5)
β -thalassaemia	0	16	(0)	5 (3)	9	(21.4)
Normal Hb	10 (8)	126	(5.9)	29 (19)	95	(15.3)

The figures in brackets indicate the number and percentage of individuals expected with falciparum infection.

TABLE 4. THE COMPARISON OF *P. FALCIPARUM* PARASITE DENSITIES IN HAEMOGLOBINOPATHIES

Subjects	1-999	%	1000-9999	%	10000-49999	%	50000-99999	%	100000+	%	Total
AE	a	7	2	27	10	24	5	12	7	17	41
	b	1	9								
α -thalassaemia	a	3	1	29	2	12	2	12	5	29	17
	b		4								
β -thalassaemia	a	1	2	20	3	30	1	10	2	20	10
	b	1									
Normal	a	16	6	14	5	39	4	7	7	12	57
	b		2								

The figures in upper line (a) and lower line (b) in each column indicate frequency with corresponding parasite density in the surveyed and hospital samples.

REFERENCES

- Das Gupta, G. R. et al. (1956) International Society of Haematology, Sixth International Congress, New York, Grune & Stratton 1958, p. 733
- Flatz, G., Pik, K. & Sumdharagiati, B. (1964) Lancet, ii, 385
- Huisman, T. J. H. & Dozy, A. M. (1961) Analyt. Biochem., 2, 400
- Kruatrachue, M., Na-Nakorn, S. & Charoenlarp, P. (1960) Proceedings of the Eighth International Congress of Haematology, Japan, Pan Pacific Press
- Kruatrachue, M., Klongkamnuankarn, K. & Harinasuta, C. (1966) Lancet, i, 404
- Kruatrachue, M. et al. (1962) Lancet, ii, 1183
- Kunkel, H. G. & Wallenius, G. (1955), Science, 122, 288
- Lie-Injo Luan, Chin, J. & Ti, T. S. (1965) Ann. hum. Genet., 28, 173
- Na-Nakorn, S., Minich, V. & Chernott, A. I. (1956) J. Lab. clin. Med., 47, 490
- Pootrakul, S., Wasi, P. & Na-Nakorn, S. (1966) Abstracts of papers, Eleventh Congress of the International Society of Hematology, Sydney, p. 123
- Schade, A. L., Reinhart, J. & Miller, J. R. (1954) Proc. Soc. exp. Biol., 87, 443
- Singer, K., Chernoff, A. I. & Singer, L. (1951) Blood, 6, 413
- Tuchinda, S. et al. (1964) Amer. J. hum. Genet., 16, 311
- Wasi, P., Na-Nakorn, S. & Suingdumrong, A. (1964) Nature, 204, 907
- Wasi, P., Na-Nakorn, S. (1966) J. med. Ass. Thailand, 49, 757
- World Health Organization (1966) Hemoglobinopathies and allied disorders, Wld Hlth Org. techn. Rep. Ser., 338, 28

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