The haemoglobin pattern of sickle cell and haemoglobin C β^+ -thalassaemia in Liberia

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SUMMARY Haemoglobin components in 21 Liberians with Hb S β^+ -thalassaemia and four with Hb C β^+ -thalassaemia were measured to classify the forms of β^+ -thalassaemia present in the population. In 20 Hb S and all Hb C β^+ -thalassaemics the data were consistent with the interaction of these variants with the mild type 2 (Negro) form of β^+ -thalassaemia. The data available were insufficient to classify the remaining case, a young child. It was concluded that the clinically more severe type of β^+ -thalassaemia giving Hb A levels of 5 to 15% in compound heterozygotes is probably uncommon in Liberia.

Analysis of haemoglobin A levels in double heterozygotes for β -thalassaemia and β chain structural haemoglobin variants is important in classifying the different forms of β^+ -thalassaemia present in a population.¹ Previous studies have shown there to be two main types of Hb S β^+ -thalassaemia, those with haemoglobin A in the 5 to 15% range and those with levels of 20 to 30% haemoglobin A. The former is usually associated with a more severe clinical course, whereas the latter normally results from the interaction of haemoglobin S with the mild type 2 (Negro) form of β -thalassaemia.

Sickle cell β^+ -thalassaemia is well documented in black populations.²⁻⁴ However, there are few published data relating to the quantification of haemoglobin A in Hb S β^+ -thalassaemia in Negroes other than from the USA or Jamaica.¹

In Liberia there is a high frequency of both haemoglobin S and β -thalassaemia,^{5,6} and compound heterozygotes for β -thalassaemia and haemoglobin S are encountered not infrequently. In this report, data are given on the levels of haemoglobin A in double heterozygotes for β^+ -thalassaemia and haemoglobins S or C from both clinical work and surveys.

Materials and methods

The subjects lived either in the Mount Nimba area of northern Liberia or in Buchanan on the coast of central Liberia. However, many originated from

other parts of Liberia and several ethnic groups are represented. Date of birth data were not available in most instances and so ages were mainly estimated.

The diagnosis of sickle cell β^+ -thalassaemia was arrived at by demonstrating haemoglobins S, A₁ and A₂ on electrophoresis. Standard methods of haemoglobin electrophoresis and identification of haemoglobin components were used.⁷ Haemolysates were prepared in most instances from venous blood; occasionally fingerprick blood was used. Screening haemoglobin electrophoresis was done on cellulose acetate using a Tris buffer system pH 8.5. A solubility test was used to confirm the identity of haemoglobin components migrating as Hb S. There was good separation of haemoglobins A+F, S, and A₂ or C. These were measured by elution following electrophoresis.⁸ Hb A concentrations were calculated by subtracting the Hb F values obtained by alkali denaturation⁹ from the Hb A+F fraction measured by elution. In this laboratory the normal range of Hb A₂ is 1.5 to 3.2%. β -thalassaemia trait carriers normally have a value over 3.7%.⁵ The intracellular distribution of Hb F was studied by an elution method using a citric acid buffer.¹⁰

Results

Twenty-one sickle cell β^+ -thalassaemia (table) and four Hb C β^+ -thalassaemia compound heterozygotes were identified during investigations on over 10 000 people. Sickle cell β^0 -thalassaemia was not observed. Nine sickle cell and three Hb C β^+ -thalassaemics were incidental findings during population screening, health checks for employment,

TABLE Haemoglobin analysis and summary of clinical findings in 21 Liberian double heterozygotes for haemoglobin S and β^+ -thalassaemia.

Case No	Tribe	Age (yr)	Sex	Haemoglobin analysis (%)				Clinical findings
				A	F	S	A ₂	
1	Bassa*	1	F	25.7	5.3	65.0	4.0	Anaemia, splenomegaly
2	Mano*	1	F	31.3	(A+F)†	62.5	6.2	Anaemia, splenomegaly
3	Bassa	3	M	25.8	2.7	65.8	5.7	Anaemia, hepatosplenomegaly
4	Gissi	6	M	6.5	24.2	66.7	2.6	Anaemia, splenomegaly
5	Mano*	8	M	25.6	5.7	64.0	4.7	Anaemia, splenomegaly
6	Bassa	10	F	22.8	5.1	67.2	4.9	Anaemia, splenomegaly
7	Bassa	10	F	24.5	3.4	68.5	3.6	Anaemia, splenomegaly
8	Mandingo*	15	F	17.0	16.7	62.9	3.4	Mild anaemia
9	Mano	15	F	18.8	9.2	68.0	4.0	Anaemia, pregnant
10	Bassa	15	M	30.0	5.4	60.0	4.6	Mild anaemia
11	Bassa	18	F	24.9	5.1	65.0	5.0	Anaemia, pregnant
12	Bassa	18	M	15.5	18.2	63.8	2.5	Mild anaemia
13	Bassa	18	F	22.0	1.0	72.0	5.0	Anaemia, pregnant
14	Gio	18	F	24.3	5.9	64.8	5.0	Mild anaemia, leg ulcers
15	Kpelle	Adult	M	27.0	<1.0	68.0	4.1	Mild anaemia
16	Bassa	Adult	F	16.2	10.0	68.5	5.3	Severe anaemia, pregnant
17	Bassa*	Adult	F	24.1	20.1	52.3	3.5	Mild anaemia
18	Bassa	Adult	F	30.0	<1.0	63.6	6.4	Post-partum haemorrhage
19	Gissi	Adult	M	31.0	9.0	54.5	5.5	Mild anaemia
20	Bassa	Adult	M	29.4	2.4	64.0	4.2	Mild anaemia
21	Bassa	Adult	M	25.3	1.4	69.0	4.3	Mild anaemia

*Diagnosis supported by family studies.

†No separate Hb F determination.

blood donation, etc. Among the remainder, in only two instances was a haemoglobinopathy suspected as the primary diagnosis. In children with splenomegaly, the degree of enlargement was not greater than that seen in normal children from the same areas of holoendemic malaria.

In the 20 cases where full haemoglobin analysis was done (table), Hb S β^+ -thalassaemics fell into two groups; 16 subjects had a haemoglobin A level (within experimental limits) in the 20 to 30% range (mean 25.7%, SEM $\pm$ 0.81%). Hb A₂ levels were raised (mean 4.6%, SEM $\pm$ 0.2%). One woman (case 17) with an unusually high Hb F level (20%) has been described previously. She had one child with homozygous β^+ -thalassaemia and another with sickle cell trait.¹¹ The mean Hb F value of the other 15 subjects was 4.2% (SEM $\pm$ 0.7%).

In cases 8, 12, and 16, the comparatively low Hb A value (15.5 to 17.2%) was associated with a fetal haemoglobin of 10% or greater. Case 12 had a Hb A₂ level in the normal range. A further case with a high Hb F level, a young child (case 4), had 6.5% Hb A and Hb A₂ in the normal range. His mother was not available for investigation but the alleged father had a normal haemoglobin pattern. In all Hb S β^+ -thalassaemics fetal haemoglobin showed a heterocellular distribution.

Hb A levels in the four Hb C β^+ -thalassaemics were 20.1, 23.4, 26.4, and 28.0%. The highest level of Hb F was 5.2% and the distribution of Hb F was

heterocellular. One of the Hb C β^+ -thalassaemics, an adult male, was anaemic with mild hepatosplenomegaly. The other three, two of them pregnant women, were mildly anaemic.

Discussion

The results indicate that although there was some heterogeneity, most cases of sickle cell and Hb C β^+ -thalassaemia described here resulted from the interaction of these variants with the type 2 (Negro) form of β^+ -thalassaemia. In the three older subjects in whom high Hb F levels were associated with a slightly low Hb A value, a heterocellular HPFH gene may have been segregating, as suggested for the 18-year-old Negro boy with Hb S β^+ -thalassaemia described by Weatherall.² However, in these three patients, genetic evidence is lacking and it cannot be excluded that the high levels of Hb F caused some artefactual difficulty in estimating the level of Hb A.

An associated HPFH gene could also explain the findings in the Gissi boy with a very low Hb A value. However, here the high Hb F level can be explained on the basis of age and he could have had the low Hb A type of Hb S β^+ -thalassaemia, but again without genetic evidence no firm conclusions can be reached.

The normal Hb A₂ values seen here in cases 4 and 12 are unusual in Hb S β^+ -thalassaemia in black populations, although this has been reported in association with high Hb F levels in other racial

groups.¹ However, in the Jamaican series of Serjeant *et al.*,⁴ two persons with the Negro type of Hb S β^+ -thalassaemia had Hb A₂ values in the normal range.

In earlier reports of Hb S β^+ -thalassaemia in black populations from the USA² and Jamaica,⁴ subjects with haemoglobin A in both the 5 to 15% and 20 to 30% ranges were described. However, the β -thalassaemia gene in the former cases usually came from a non-black source. By contrast, a previous study of β -thalassaemia homozygotes in Liberia¹¹ suggested, as here, that the type of β^+ -thalassaemia giving Hb A levels of 5 to 15% in compound heterozygotes is uncommon in Liberia.

How frequent is sickle cell β^+ -thalassaemia in Liberia? Although the syndrome was found in about two per 1000 persons, this was a biased sample, being partly based on a hospital population. However, the Bassa tribe from which most of the subjects in this study came has been shown to have a high frequency of both haemoglobin S and β -thalassaemia.^{5 6 12} Based on gene frequencies of 0.041 for Hb S and 0.067 for β -thalassaemia,¹³ the estimated frequency of double heterozygotes in the Bassa comes out at more than five per 1000. It is unlikely to be as high as this, however, as the two genes do not appear to be evenly distributed among the Bassa.^{5 14}

Nevertheless, sickle cell β^+ -thalassaemia is clearly not uncommon in Liberia and although usually mild clinically may present more severe manifestations during pregnancy. Unfortunately, as with other thalassaemia syndromes, detailed haemoglobin studies are needed to characterise the condition. As these are often not available in countries like Liberia, sickle cell β -thalassaemia will usually go undetected.

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