

Thalassaemia and haemoglobinopathies in Brunei Darussalam

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Summary

One thousand consecutive Brunei Darussalam patients referred with low Hb, and/or low MCV and MCH (Hb<12.5g/dl, MCV<76fl, MCH<27pg) were studied in the laboratory for underlying haemoglobinopathies. 30.0% of such patients were proved to have either β – thalassaemia trait, β – thalassaemia major, Hb AE, Hb EE, Hb E β – thalassaemia or Hb H disease. In some, the haemoglobin abnormality was not identified precisely. α – thalassaemia was suspected in an additional 4.3% of cases but confirmation study by globin-chain synthesis was not available. β – thalassaemia trait which was the predominant disorder was equally distributed among the three major race groups of Brunei Darussalam. Hb E was found exclusive among the Malay population. Hb H disease appeared as more common among the Chinese or the Malays ($p>0.05$).

This study reveals that thalassaemia and haemoglobinopathies are prevalent in Brunei Darussalam.

Key words: thalassaemia, haemoglobinopathies.

Introduction

Thalassaemias and haemoglobinopathies are widely prevalent in South-East Asia. Numerous studies of these blood disorders are available from the various countries of this region.¹⁻⁷ To the best of the knowledge of this author, no large scale study of such diseases has been previously reported from Brunei Darussalam. We report the prevalence of these disorders in a selected Brunei Darussalam population.

Patients and Methods

During a 17 months period from January 1985 to May 1986, one thousand consecutive samples from all Hospitals and Clinics originating from the three main racial groups of Brunei Darussalam were studied. The patients referred from investigation had either anaemia (Hb<12.5g/dl), or low MCV and MCH(<76fl and <27pg). 551 (55.1%) of such referrals were from antenatal clinics. The characteristics of the patients studied are summarised in Table I.

The Brunei Darussalam population is composed of approximately 65% Malays, 20% Chinese and 10% indigenous tribes.⁸ The remaining 5% are not native to Brunei Darussalam and such persons were excluded from the present study.

In all cases a Leishman stained blood smear was examined for red cell morphology and supravital staining done to detect inclusion bodies. Haematological indices were measured using the Coulter Counter Model S standardised with cell control (4C) provided by Coulter Electronics. Haemoglobin

Table I
Distribution of ages in 1000 patients

Race	Sex	No. cases	Age Groups							Mean age
			1-10	11-20	21-30	31-40	41-50	51-60	61-70	
Malay	Male	191	96	14	39	17	11	5	9	18.9
	Female	613	78	77	302	109	26	11	10	24.6
Chinese	Male	30	5	2	11	6	0	5	1	28.9
	Female	80	10	5	39	17	6	3	0	28.3
Indige- nous Tribes	Male	18	10	3	2	1	1	1	0	15.2
	Female	68	4	4	36	16	3	4	1	25.8

electrophoresis was done using cellulose acetate strips (Titan III H – Helena) at pH 8.2 – 8.6. Any unusual haemoglobin variants detected were identified by further electrophoresis on alkaline agarose gel (pH 8.6) and acid agarose gel (pH 6.0) using Paragon electrophoresis system. Hb A2 and Hb E were quantitated using Helena Beta – Thal Hb A2 Quik Column Kit. HB F was measured by the alkaline resistance method of Singer.⁹ Serum iron was quantitated using the bathophenanthroline method without deproteinisation (Iron BP Test – Roche) and total iron binding capacity estimated by the precipitation method with magnesium hydroxide (IBC Test – Roche).

The principal laboratory data used to define various haemoglobinopathies are :

- (1) β – thalassaemia trait, peripheral blood film shows presence of hypochromasia, aniso-poikilocytosis, and haemoglobin A2 values over 3.6% (Reference range at RIPAS laboratory is 2.2 – 3.5%);
- (2) β – thalassaemia major, blood picture shows marked hypochromasia, aniso-poikilocytosis, reticulocytosis, target cells, normoblasts and the haemoglobin pattern consists almost entirely of Haemoglobin F;
- (3) Haemoglobin E trait – presence of mild hypochromia and microcytosis with Haemoglobin E values ranging from 23 – 30 percent;
- (4) Haemoglobin E disease, the major red cell morphologic feature is the presence of numerous target cells and haemoglobin pattern consists almost entirely of haemoglobin E;
- (5) Haemoglobin E β – thalassaemia – has similar haematologic picture as homozygous β – thalassaemia and haemoglobin analysis shows only Haemoglobins E and F;
- (6) Haemoglobin H disease – the characteristic intraerythrocytic inclusion bodies present as demonstrated by supra-vital staining and typical H band on cellulose acetate electrophoresis.

Results

Of the one thousand patients studied there were 343 (34.3%) with thalassaemia or haemoglobinopathy. The distribution of these blood disorders among the three races is shown in Table II.

β – thalassaemia trait was the predominant disorder detected in this study accounting for 22.7% of all abnormals. The haematological indices in this group is summarised in Table III. Peripheral blood smears showed microcytosis with mild to moderate hypochromasia, anisocytosis and target cells. Basophilic stippling was sometimes observed. The anaemia was relatively mild with a mean haemoglobin of 10.6 ± 1.8 (SD). Mean MCV was 67.4 ± 7.5 . MCH was characteristically low with a mean of 21.9 ± 2.8 .

Table II
Distribution of thalassaemia and haemoglobinopathies by race and sex

Race	β -Thalassaemia trait	β -Thalassaemia major	Hb AE	Hb EE	Hb E- β thalassaemia	Hb H disease	α -thalassaemia trait	Total for each race
Malay	183	3	37	5	8	10	31	277 (80.7%)
Chinese	31	1	0	0	0	7	11	50 (14.6%)
Indigenous tribes	13	0	0	0	0	2	1	16 (4.7%)
Total	227	4	37	5	8	19	43	—

The 4 cases of β – thalassaemia major detected in the study were clinically symptomatic. Three had hepatosplenomegaly and all of them had haemoglobin below 7.4. Peripheral blood smears showed marked hypochromia, poikilocytosis, target cells and nucleated erythrocytes. The reticulocyte count was elevated in all four patients. Bone marrow examination in two of these cases showed erythroid hyperplasia with significant dyserythropoiesis. One of the patient in addition showed severe megaloblastic erythropoiesis consequent to folic acid deficiency.

Table III
Haematological indices in β -thalassaemia trait, Hb E trait and Hb H disease

	Laboratory Reference Range	β -thalassaemia trait \pm SD	Hb E trait \pm SD	Hb H disease \pm SD
Hb (g/dl)	12.5 – 17	10.6 \pm 1.82	11.1 \pm 2.19	9.2 \pm 2.15
RBC ($\times 10^{12}/l$)	4.2 – 6.2	4.9 \pm 0.91	4.8 \pm 0.79	5.1 \pm 0.83
PVC (%)	39 – 51	33.1 \pm 5.35	32.7 \pm 8.99	32.5 \pm 6.10
MCV (fl)	76 – 96	67.4 \pm 7.46	72.0 \pm 9.70	65.0 \pm 6.34
MCH (pg)	27 – 32	21.9 \pm 2.85	23.8 \pm 4.26	19.0 \pm 1.74
MCHC (g/dl)	30 – 36	32.3 \pm 1.64	32.6 \pm 1.83	29.2 \pm 1.54
Hb A2 (%) or HbA2 + HbE (%)	2.2 – 3.5	5.3 \pm 1.15	25.6 \pm 4.79	1.3 \pm 0.58
Hb F (%)	0.5 – 1.7	2.4 \pm 2.18	0.9 \pm 0.84	1.2 \pm 0.83
Serum Fe (μ mol/l)	10 – 30	23.1 \pm 11.85	24.3 \pm 14.94	37.3 \pm 14.62
Serum TIBC (μ mol/l)	45 – 70	65.9 \pm 21.05	78.4 \pm 19.06	57.4 \pm 15.84

Heterozygous Hb E was detected in 37 patients. All these patients were of Malay origin. The five cases of homozygous Hb E were also seen exclusively in Malays. The haematological indices in heterozygous patients showed only minor abnormalities and all were clinically asymptomatic. The mean Hb E was 25.6 ± 4.8 and Hb F was 0.9 ± 0.8 . Serum iron was in the upper limits of the normal range with a mean of 24.3 ± 14.9 . Total iron binding capacity was slightly above the normal range with a mean of 78.4 ± 19.0 . Homozygous Hb E showed haematological indices within the normal range. Hb E was considerably raised with a mean of 80.6 ± 3.4 and Hb F showed a mean of 1.4 ± 1.3 . Peripheral blood smear examinations showed erythrocytes which were almost exclusively target cells. Thus the homozygous state for Hb E can be distinguished without difficulty from the heterozygous carrier by examination of peripheral blood, the very high Hb E, and total absence of Hb A in cellulose acetate electrophoresis. None of these four patients had any clinical symptoms.

Hb H disease diagnosed in nineteen cases was found in all three ethnic groups. The majority were symptomatic and nine patients had splenomegaly. On cellulose acetate electrophoresis care was taken to read the bands as soon as possible (within 5 minutes) as small amounts of Hb H may disperse beyond this period. In all cases supravital examination of patients blood showed the characteristic red cell inclusions. Peripheral blood smears showed marked hypochromia and aniso-poikilocytosis. Target cells were abundant. The mean haemoglobin was 9.2 ± 2.1 and other parameters were also below normal range. Serum iron had a mean of 37.3 ± 14.6 . Bone marrow examination done in 3 cases showed erythroid hyperplasia with megaloblastic erythropoiesis in one patient.

43 cases in this study showed microcytosis with a low MCV, low MCH, normal MCHC and normal serum iron. Haemoglobin electrophoresis did not reveal any abnormal bands. Quantitation of Hb A₂ showed results which were either normal or slightly below normal. Out of these forty-three cases, five samples showed the presence of inclusion bodies. Therefore, these five cases are those of α - thalassaemia trait. But we cannot exclude the other thirty-eight cases, since it needs further investigation by analysis of the globin chain synthesis ratio. Unfortunately we are unable to do this investigation at RIPAS Hospital laboratory.

Discussion

The present study, the first of its kind from Brunei Darussalam, has attempted to define the prevalence of thalassaemia and haemoglobinopathies in a selected group of its native population. The 'select' group comprised a population in whom an underlying haemoglobinopathy was suspected for a clinical reason or, more often, where initial laboratory findings suggested a blood dyscrasia likely to be due to a haemoglobin abnormality. It is stressed that the present findings do not reflect the prevalence of these diseases in the general population which, however, will be the subject of a forthcoming study.

300 (30.0%) of one thousand patients of Brunei Darussalam origin referred to the laboratory were proven to have thalassaemia or haemoglobinopathy. The majority were asymptomatic and the only clue to some haematological defect was the laboratory demonstration of unexplained mild anaemia, or minor abnormalities in red cell parameters. In an area such as South-East Asia where haemoglobinopathies of one type or other are widely prevalent, it is appropriate that such 'minor' red cell abnormalities be subjected to a thorough laboratory examination so that any underlying inherited defect of haemoglobin may be excluded and genetic counselling can be given.

β - thalassaemia trait was diagnosed in 22.7% of this population and accounted for over 66% of all haemoglobinopathies detected. The disparity between a near normal haemoglobin and low MCV in these patients deserves special attention and has been previously remarked upon by other investigators.¹⁰ The large proportion of antenatal patients in this study in whom 116 were proven to have β - thalassaemia trait but who were all clinically asymptomatic reiterate the need for a thorough investigation of 'minor red cell abnormalities' in order that genetic counselling can be made the most effective.¹¹⁻¹² No significant increase in the prevalence of β - thalassaemia trait was found among the three ethnic groups in this study.

Hb E disease in both the heterozygous and homozygous states was found exclusively among the Malay race. The high prevalence of this disease among the Malays is in keeping with other studies from this region.¹³ None of the patients was clinically symptomatic.

Hb H diseases was diagnosed in nineteen patients and the majority were clinically symptomatic. In six of these patients, there was an associated splenomegaly. The haematological indices have been described. The disease was seen in all three ethnic groups and appeared to be more frequent among the Chinese but did not prove significant when compared to the Malays ($p > 0.05$).

Forty-three patients had microcytosis with low MCV and MCH but normal MCHC and serum iron. Haemoglobin electrophoresis did not reveal any abnormal bands. Hb A₂ was normal or slightly below normal. These are probably patients who are α -thalassaemia carriers. A definitive diagnosis of these cases requires a more elaborate study of globin synthesis ratio and as presently we are yet to incorporate this technology into our laboratory.

In summary, this study⁷ has attempted to categorise and classify the prevalence of various haemoglobinopathies in our country in a selected population. This will serve as guidance to a forthcoming general population survey. It also emphasises the need for detailed investigation of minor unexplained deviations in red cell indices in an area where haemoglobinopathies are widely prevalent. The importance of such extended investigations is borne out in this study which included a large, clinically asymptomatic antenatal population in whom genetic counselling may be required and iron therapy is not given to them as their iron status is normal.

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