Mild Homozygous High A2-Type Beta Thalassaemia

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THE HETEROGENEITY OF beta-thalassaemia has been pointed out by several investigators.[1-5] The classic high A2 type of β-thalassaemia is far more common than others and when unspecified this type of thalassaemia is usually referred to. The homozygous form of this disease usually gives the classical clinical picture of thalassaemia major (Cooley's anaemia), described by Cooley & Lee in 1955, which is usually characterised by severe anaemia which is insidious in onset and usually obvious within the first two years of life and often from about the third month of life. This is associated with hepato-splenomegaly and bone changes; the latter is due to marrow hypercellularity and results in a mongoloid facies with X-ray changes best seen in the skull and in the tubular bones of the extremities; and there is usually general retardation of growth. Other clinical features which may be present include jaundice, periodic attacks of fever, cardiac symptoms secondary to the anaemia and occasionally leg ulcers and hypogonadism in those who survive up to puberty. The haemoglobin is usually between 3 and 9 g/100 ml. The red cells show marked anisopoikilocytosis and hypochromia with often many cell fragments and target cells. Variable numbers of reticulocytes and nucleated red cells are present. The mean corpuscular volume and the mean corpuscular haemoglobin are significantly reduced. The osmotic fragility test characteristically reveals an increased resistance to haemolysis and the serum bilirubin is usually slightly raised.

Homozygous β-thalassaemia is usually fatal in childhood although a few patients with this disorder have been reported to survive beyond the fourth decade of life.[6-9] The present report describes a patient in Kuala Lumpur, with homozygous high A2 type of β-thalassaemia who started having clinical symptoms for the first time only in the third decade of life and who clinically had only very mild manifestations of the disease.

Methods

Haematological examinations were carried out according to standard methods.[10] Haemolystaes were prepared from washed packed red cells by the addition of 1 volume of water and 0.5 volume of toluene. Haemoglobin F levels were measured by the method of Singer *et al.*[11] The distribution of haemoglobin F in the red cells was examined by the acid elution technique of Kleihauer.[12] Electrophoresis of haemoglobin was done on starch gel using tris – EDTA boric acid buffer at pH 8.6 and discontinuous tris boric acid buffer at pH 9.5. Agar gel electrophoresis was done in citric acid buffer at pH 6.9 (Robinson *et al.* 1957).[13] Cellulose acetate electrophoresis was done in tris – EDTA boric acid buffer at pH 8.9. Haemoglobin A2 was quantitated by the cellulose acetate electrophoretic method of Marengo-Rowe.[14]

Case Report

The *propositus* was a 22-year old male Chinese who was admitted to the Assunta Hospital on 1.6.73 complaining of yellowness of his eyes for the last three months. The day before admission he began to have fever and felt weak. On questioning he admitted to his urine being yellow throughout. On examination he was found to be of average height. But he was pale and slightly jaundiced. His spleen was felt 6 cm. below the costal margin and his liver

1 cm. below the costal margin and both were firm and non-tender. He did not have any ascites or oedema and there were no other stigmata of liver disease. At the time of admission the patient had a temperature of 100°F and the temperature came down to normal five days after admission.

Laboratory findings: The haematological findings for the patient and his family are shown in Table I. The peripheral blood film (Fig. 1) showed mild hypochromia and moderate anisopoikilocytosis with some microcytes, target cells and schistocytes. Haemoglobin analysis showed 40.3% Hb F and 9.1% Hb A2. Haemoglobin electrophoresis was done on cellulose acetate, starch gel at pH 8.6 and 9.5 and on agar gel and the patient was seen to have haemoglobin A together with haemoglobin F and increased amounts of Hb A2. Starch gel electrophoresis at pH 8.6 is shown in Fig. 2 and agar gel electrophoresis in Fig. 3. The distribution of Hb F in the red cells was heterogeneous. The white cell count was 9,350 with 28% neutrophils, 18% stab cells, 45% lymphocytes, 6% monocytes, 2% eosinophils and 1% basophils. The platelet count was 310,000/cu. mm. The total serum bilirubin was 3.5 mg/100 ml. with 0.6 mg.% direct reacting and 2.9 mg.% indirect reacting. Urobilinogen was increased in the urine and there was a trace of bilirubin. The total serum protein was 6.0 mg.% with 2.1 gm.% of globulin. The alkaline phosphatase was 5.4 K. A. units. No haptoglobin was visible on starch gel electrophoresis. The Coomb's test was direct and indirect negative. Motulsky's test for G6PD deficiency was normal.

X-ray skull showed no abnormal bone changes.

Pedigree of family

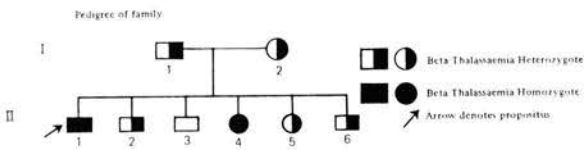

Table I
Haematologic values in predigree

	Age (yr)	Hb (g/100 ml)	RBC mill/cu. mm.	PCV %	MCV	MCH	MCHC	Reticulocytes %	Red cell morphology	Osmotic fragility	Hb. F %	Hb. A2%	Serum iron (ug/100 ml)
Father (I-1)	45	15.2	5.8	50	86.2	26.2	30.4	0.8	±	normal	2.3	6.4	—
Mother (I-2)	45	10.0	4.4	35.5	80.7	22.7	28.2	1.8	+	normal	2.4	6.0	—
Propositus (II-1)	23	10.3	5.4	35	64.8	19.1	29.4	2.5	++	decreased	40.3	9.1	150
Brother (II-2)	20	13.0	4.9	41	83.7	26.5	31.7	1.8	±	normal	2.6	6.0	150
Brother (II-3)	17	13.1	4.3	41.5	96.5	30.5	31.5	0.8	0	normal	1.8	2.9	—
Sister (II-4)	14	7.6	3.3	23.5	71.2	23	32.3	10.2	+++	decreased	55.5	4.2	229
Sister (II-5)	9	12.0	4.5	38	84.4	26.7	31.6	1.6	±	normal	2.4	6.1	135
Brother (II-6)	7	12.0	4.3	38	88.4	28	31.6	0.4	±	normal	2.4	6.3	113

± = very mild abnormalities
\+ = mild abnormalities
++ = moderate abnormalities
+++ = severe abnormalities

Family studies: One sister (II-4) of the propositus aged 15 years gave a of having been unwell and of having had slight jaundice off and on frequently since 7 years of age. She has been attending the follow-up clinic at the General Hospital, Kuala Lumpur but has never had any blood transfusion. When seen by us on 3.7.73 ,her height was seen to be within normal limits for her age but she had mongoloid facies and was slightly jaundiced

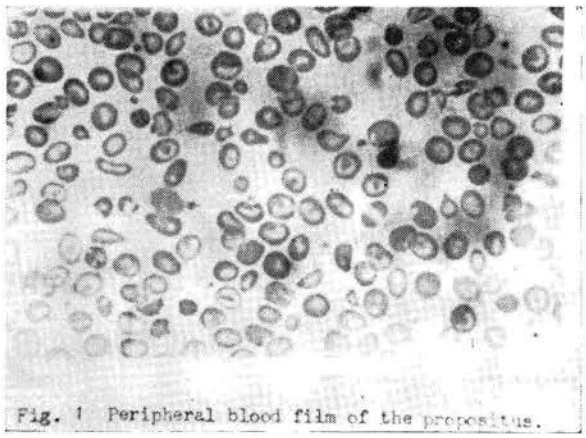

Fig. 1 Peripheral blood film of the propositus.

with sallow skin. The spleen was felt 7 cm. below the costal margin and the liver 3 cm. below the costal margin. The laboratory findings in this sister are also listed in Table I. Her peripheral blood film showed marked anisopoikilocytosis with many microcytes and macrocytes together with many schistocytes and irregularly contracted cells. Haemoglobin analysis showed 55.5% Hb. F and 4.2% Hb A_2. Haemoglobin electrophoresis was again done on cellulose acetate, starch gel at pH 8.6 and 9.5 and on agar gel and this sister (II-4) was also seen to have haemoglobin A together with haemoglobin F and increased amounts of A_2. The distribution of Hb F in the red cells was heterogeneous. The white cell count was 10,250 with 30 neutrophils, 23 stab cells, 2 myelocytes, 31 lymphocytes, 1 monocyte, 1 eosinophil and 12 nucleated red cells per 100 white cells. The platelet count was 250,000/cu.mm. The serum bilirubin was 3.9 mg/100 ml. No haptoglobin was visible on starch gel electrophiresis. Motulsky's test for G6PD deficiency was normal.

X-ray skull showed thinning of the tables especially the outer with bony trabeculae at right angles to the tables giving rise to the characteristic hair-on-end appearance.

The parents and the other members of the family gave no history of anemia, jaundice or of any illness of note and were quite well. They were all examined in the same manner as the propositus (II-1) and his sister (II-4) and both the parents of the propositus and two of his brothers (II-2 and II-6) and one sister (II-5) were seen to have elevated Hb A_2 values and slight morphological abnormalities on peripheral blood smear consistent with a diagnosis of β-thalassaemia trait, although the mean corpuscular volume was normal in all of them. One brother (II-2) was haematologically normal.

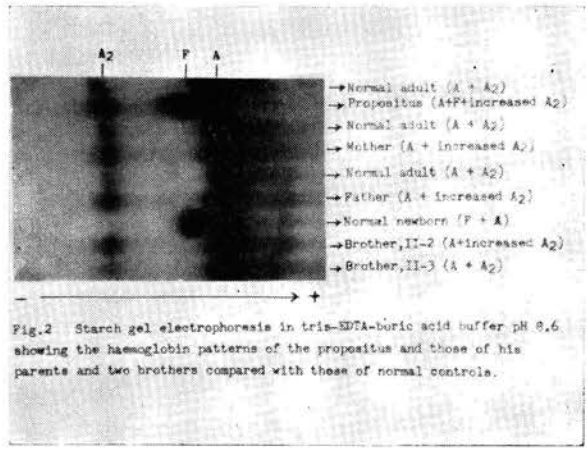

Fig.2 Starch gel electrophoresis in tris-EDTA-boric acid buffer pH 8.6 showing the haemoglobin patterns of the propositus and those of his parents and two brothers compared with these of normal controls.

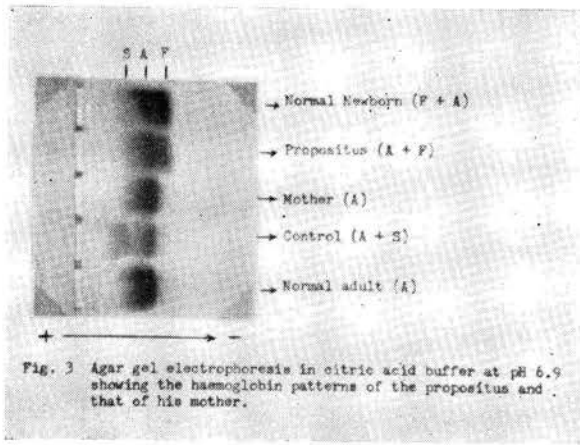

Fig. 3 Agar gel electrophoresis in citric acid buffer at pH 6.9 showing the haemoglobin patterns of the propositus and that of his mother.

Discussion

The patient in this study showed the symptoms of homozygous high A_2 type of β-thalassaemia but with unusually mild clinical features. Unlike the classical case of Cooley's anemia, our patient (II-1) started having symptoms only at 22 years of age and when examined his haemoglobin level was found to be relatively good (10.3 gm/100 ml.). In this family however, there is a significant difference in the severity of the clinical and haematological manifestations of the propositus (II-1) and his similarly affected sister (II-4). The latter appears to have had symptoms at least from 7 years of age.

But even the latter is still fairly well and attending school and has never had any necessity for blood transfusion. The clinical and haematological features in our patient are therefore consistent with the diagnosis of the so-called thalassaemia intermedia,[7] a clinical syndrome which is intermediate in severity between the mild abnormalities found in β-thalassaemia trait and those found in patients with homozygous β-thalassaemia or thalassaemia major. In most cases of thalassaemia intermedia, anaemia, hepatosplenomegaly, skeletal changes and elevated Hb F levels are present to varying degrees and many patients with this syndrome do not require significant transfusion therapy. In such patients the diagnostic possiblities that have to be considered include: (1) double heterozygosity for hereditary persistance of fetal haemoglobin (HPF) gene and a β-thalassaemia gene; (2) double heterozygosity for two different variants of β-thalassaemia, high A2 and high F β-thalassaemia (β ≷ thalassaemia); (3) β-thalassaemia trait alone; (4) combination of β-thalassaemia with α-thalassaemia; (5) true homozygosity for a mild type of high A2 β-thalassaemia. In family A, both the parents have the high A2 type of β-thalassaemia trait with no evidence for the HPF gene or high F β-thalassaemia gene. Therefore the first and second diagnostic possibilities of a double heterozygosity cannot occur in the propositus. The third possibility of whether the propositus (II-1) and his sister (II-4) are merely having β-thalassaemia trait alone has also to be considered. This however, appears to be unlikely when the clinical and haematological findings and the levels of Hb F and A2 in the propositus and his sister are compared with those of the other members of the family. One can however, only say this with certainty if the propositus or his sister marry a normal individual and have all their children showing β-thalassaemia trait. The possibility that the condition in our patient is the result of an interaction between α and β thalassaemia genes as was thought to be the case in the patients described by Fessas (1961[15], 1965[16]), by Pearson (1966)[17] and by Kan and Nathan (1970)[18] cannot be entirely ruled out. It is assumed that the presence of α-thalassaemia tends to ameliorate the condition of β-thalassaemia, the lack of α chains being more or less compensated by a lack of β chains resulting in less imbalance of available α and β chains. Kan and Nathan (1970) suggested from the results of chain synthesis studies that one of their cases of mild Cooley's anaemia was a combination of two β-thalassaemia genes each one received from one of the parents and an additional α-thalassaemia gene received from one of the parents (α and β thalassaemia genes are not allelic). This last mentioned parent was presumably doubly heterozygous for α-thalassaemia and β-thalassaemia, a condition not leading to clinical symptoms, but resulting in an increase of Hb A2 and hypochromia and microcytosis of the red blood cells; in such a case the β/α ratio of chain synthesis is near normal instead of the ratio of around 0.5 usually found in β-thalassaemia trait. The condition in their patient with mild Cooley's anaemia with clinical symptoms is therefore presumably the result of the presence of two β-thalassaemia genes and one α-thalassaemia gene and the β/α ratio was 0.45 instead of near zero usually found in the severe cases of homozygous β-thalassaemia. However, they admitted that the finding of the β/α ratio is not an absolute proof for this assumption and that the possibility of their patient being homozygous for a mild type of β-thalassaemia cannot be entirely ruled out. Contrary to their findings, in the parents of our patients and in other members of the family with β-thalassaemia trait, the changes were very slight, suggesting a milder type of β-thalassaemia. Probably, a homozygous condition of a milder variety of high A2 type of β-thalassaemia is the most plausible explanation of the condition in our patients.

The family reported here from Malaysia shows that mild forms of homozygous high A2 type of β-thalassaemia do occur here too as have been reported in other countries. The reason for the mild clinical course in these patients may be related to further heterogeneity of the underlying defect even in the high A2 type of β-thalassaemia. Perhaps at some future date structural studies of the haemoglobin of these patients combined with studies of haemoglobin synthesis may throw some light on the exact nature of this defect. A further point to note in family A is that even within the same family two similarly affected siblings, i.e. the propositus (II-1) and his sister (II-4) can manifest different degrees of severity in the clinical and haematological features. There must therefore be other factors, genetic or environmental, which alter the expression of the β-thalassaemia genes so that different degrees in the severity of the clinical and haematological findings occur in different families and to some degree even within the same family. A careful evaluation of patients like those reported here is necessary in the search for such factors which might alter the severity of the disease. The findings at present however, show that not all cases of homozygous high A2 type of β-thalassaemia necessarily manifest the classical clinical features of severe haemolytic anaemia of early onset and an early death. Hence one must keep an open mind in considering this diagnostic possibility even when an adult presents for the first time with anaemia and hepatosplenomegaly. Also, when the diagnosis of homozygous high A2 type of β-thalassaemia is made, prognosis is not necessarily as bleak as

that which is usually associated with classical thalassaemia major (Cooley's anaemia) even in the absence of a carefully controlled transfusion schedule.

Summary

Homozygous high A2 type of β-thalassaemia with mild clinical and haematological features is described in a Chinese family in Malaysia. The report points out that such cases occur here as have been reported in other parts of the world and that the diagnosis of homozygous high A2 type of β-thalassaemia does not always carry the very gloomy prognosis that is associated with classical cases of Cooley's anaemia.

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