First observation of haemoglobin Malay α_2 B_2 26 (B1) Asn → Ser – A case report

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Summary

A new haemoglobin, Haemoglobin Malay is described in a 22 year old Malay. Structural analysis showed a AAC → AGC mutation in codon 17, with the production of an abnormal β chain (β Malay) that has an Asn → Ser substitution at position β 19. This haemoglobin variant could not be detected by conventional procedures.

Key words: β globin mutation, haemoglobin Malay, silent variant.

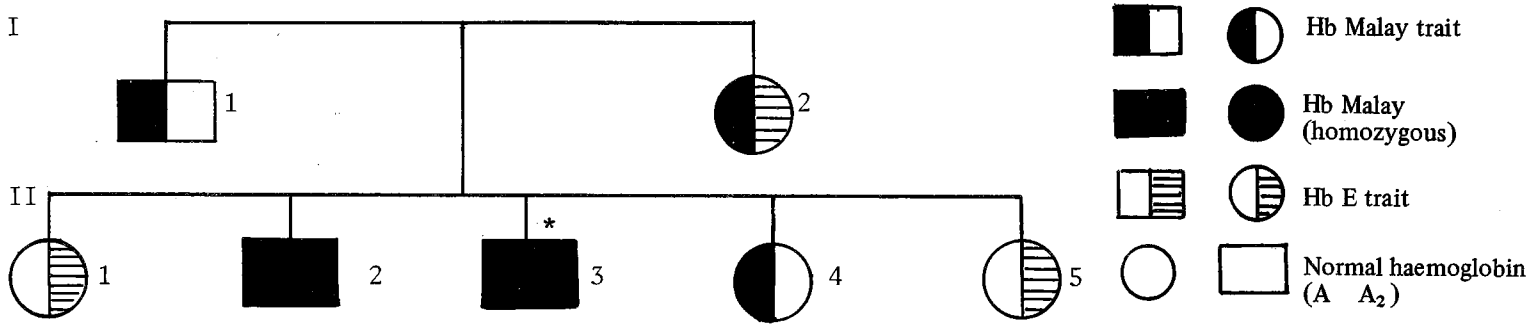
Introduction

We have recently discovered this haemoglobin in a 22 year old radiographic student from Kuala Lumpur of Malay ethnic origin.

Case report

The propositus (Figure 1 – 11.3) was referred to the Haematology Clinic, National University of Malaysia when he was found to have anaemia in a full blood picture examination. Prior to this he had been in good health and had no history of blood transfusions or hospital admissions. The physical examination revealed a young adult of normal height and weight, mild jaundice, no thalassaemic facies, and absence of hepatosplenomegaly. Examination of the peripheral blood showed moderate anaemia, moderate anisopoikilocytosis, microcytosis and hypochromia. The reticulocyte count was 2.2% and the serum ferritin level 290 ng/ml. The red cell survival using ^{51}Cr was 24 days (normal T 1/2 ^{51}Cr = 33 days). The serum bilirubin was 23 mmol/L (normal < 20 mmol/L) and the liver enzymes alanine transaminases and the alkaline phosphatase were within normal limits. Haemoglobin electrophoresis performed on starch agarose gel and on cellulose acetate Tris – EDTA borate buffer pH 8.6 and 6.0 showed bands in the normal positions of haemoglobins A, A_2 and F. Globin treated with 8 M urea and mercaptoethanol on cellulose acetate electrophoresis at pH 6.0 showed bands that correspond to the normal α, β, δ, and γ chains. The dichloroisopropanol (DCIP) and the heat test for the presence of an unstable haemoglobin were both positive. On reverse phase high performance liquid chromatography (HPLC) a haemoglobin variant that eluted slightly later than the normal β chains

Figure 1.0 Pedigree of Family AZ



Code Designation	Age	Sex	Hb	RBC	HCT	MCV	MCH	MCHC	Hb A_2	Hb F	Hb E	Hb Malay	S.B.	S.F	Diagnosis
I.1	56	M	15.8	6.2	0.5	78.5	25.4	32.3	3.4	0.4	-	+	12	120	A, Malay
I.2	50	F	9.8	4.9	0.3	63.9	20.0	31.8	-	9.7	48.1	+	30	92	E. Malay
I.1	12	F	13.2	5.3	0.4	72.2	25.1	34.8	-	0.5	28.8	-	7	26	A, E
I.2	21	M	8.6	4.6	0.3	59.7	18.8	31.5	5.0	25.2	-	+	41	320	Malay, Malay
II.3 *	22	M	9.5	5.3	0.3	56.0	18.0	32.2	6.0	10.8	-	+	28	290	Malay, Malay
II.4	27	F	11.7	5.2	0.4	67.9	22.5	33.1	3.8	0.6	-	+	7	145	A, Malay
II.5	30	F	12.3	4.9	0.4	73.1	24.7	33.0	-	1.5	32.6	-	12	84	A, E

Hb = gm/dl; RBC = 10^{12}x/L; HCT = %; MCV = fl; MCH = pg; MCHC = %; HbA_2, HbF, HbE = %; Hb Malay + = present, - not present; S.B = Serum bilirubin, mmol/L; S.F. = serum ferritin ng/ml; M = male; F = female.

was seen. Blood collected in EDTA as an anticoagulant was shipped by air for structural analysis of the haemoglobin and DNA studies at the Department of Cell and Molecular Biology, Medical College of Georgia, U.S.A. It showed a AAC → AGC mutation in codon 19 which resulted in the production of an abnormal β chain (Hbβ Malay) that has an Asn → Ser substitution at position β 19. He was homozygous for this β chain variant. Since this amino acid substitution has not been previously recorded, we designated this haemoglobin after the ethnic origin of the first case – Haemoglobin Malay.[4]

The family is of Malay extraction and their pedigree is presented in Figure 1. All siblings (Figure 1 – 11.1–11.5) were in good health prior to the study and were unaware of their inheritance of the gene for haemoglobin Malay. The sibling (Figure 1 – 11.2) had similar haematological and clinical findings as the propositus: structural analysis and DNA studies confirmed him to be homozygous for haemoglobin Malay.

Discussion

A high index of suspicion for the presence of a haemoglobinopathy lead to the discovery of this haemoglobin.

The clinical features, haematological and biochemical findings as seen in the propositus and the sibling with homozygous haemoglobin Malay are in keeping with a mild to moderate haemolytic element. Patients with thalassaemia intermedia, associated with severe ineffective erythropoiesis show increasing iron overload secondary to increased gastrointestinal absorption of iron in the absence of blood transfusions.[1] The iron overload seen may be of similar magnitude to transfusion dependent β thalassaemia homozygotes. The propositus and his sibling had serum ferritin levels of 290 ng/ml and 320 ng/ml: these levels are far below that seen in transfusion dependent thalassaemics (> 500 ng/ml). Both the cases with homozygous haemoglobin Malay studied are young adults (Figure 1 – 11.2 and 11.3) and it will be important to monitor their serum ferritin levels in subsequent years to know whether severe iron loading does occur, which may then be treated with manipulation of the diet and with the use of the chelating agent desferrioxamine (Desferal).

The two who were haemoglobin Malay heterozygotes (Figure 1 – 1.1 and 11.4) showed mild hypochromia and microcytosis with 11.4 having in addition mild anaemia.

The haemoglobin F level was normal, however, the haemoglobin A_2 level was elevated at 3.4 to 3.8%. Patients who are heterozygous for the haemoglobin Malay gene can readily (but erroneously) be identified as β thalassaemia trait as haemoglobin Malay has haemoglobin A like electrophoretic behaviour with conventional electrophoresis. The DCIP and the heat tests for the presence of unstable haemoglobin were positive in the propositus and other family members with haemoglobin Malay. Haemoglobin E ($\beta_2 B_2$ 26 Glu → Lys) is present in 3–5% of Malays.[2] These tests are usually positive with haemoglobin E. However, haemoglobin E can be readily differentiated from haemoglobin Malay, as the band runs in the same position as haemoglobin A_2 on conventional electrophoresis and the levels of haemoglobin E in the heterozygote is 30% and in the homozygote 90–100%.

Although most of the haemoglobin variants discovered to date have been detected by electrophoretic methods, these techniques fail to resolve and detect variants with neutral substitutions.[3]

Application of reverse phase HPLC for globin chain separation, peptide analysis and DNA studies were used to identify this new abnormal haemoglobin – haemoglobin Malay.

The incidence of haemoglobin Malay is unknown. Since the documentation of this first case we have been able to identify four further cases with haemoglobin Malay from amongst patients referred to our laboratory for evaluation of haematological problems.

References

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