Haemoglobin H disease and β -thalassaemia

Clinical haematological and electrophoretic studies in a family from South Lebanon*

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Summary. A family is described in which four sibs are affected with haemoglobin H disease. To our knowledge, this is the first instance where this disorder has been encountered in the Lebanon. In fact only a few cases have so far been reported from the Arab world.

All four sibs had typical haemoglobin H bands on electrophoretic examination, and characteristic intracorpuseular inclusion bodies were demonstrated in a variable proportion of their erythrocytes, as well as in cells from a younger sib and from the mother. The latter also had elevation of the Hb-A₂ fraction, and it is suggested that the above family has a combination of α - and β -thalassaemia.

Haemoglobin H disease, first described in a Chinese family by Rigas, Koler, and Osgood (1955) and independently by Gouttas *et al* (1955) in Greece, is now known to be widely distributed. It is particularly frequent in the countries of the Far East. Several cases have been reported from the Mediterranean region. In this communication we present three male and one female patients with this haemoglobinopathy, all are sibs in a family in which there may also be β -thalassaemia.

Material and methods

Both parents of the propositi originate from a small village in the southern part of Lebanon. They are Sunni Moslems and are closely related. Unfortunately our family investigation was incomplete as the household is spread over different parts of the country and could not all be contacted. The pedigree is shown in Figure 4.

Routine haematological studies were performed using standard methods (Dacie and Lewis, 1963). The alkali-resistant haemoglobin was measured by the technique of Singer, Chernoff, and Singer (1951). The abnormal haemoglobin and the A₂ fraction were determined following the procedure of Goldberg (1958). Inclusion bodies were identified after treatment of blood with brilliant cresyl blue (BCB) (Dacie and Lewis, 1963).

A mixture of anticoagulated venous blood, one part with two parts of 1% dye was incubated for 30 minutes at 37° C and then examined as smears without counterstain. At least 10,000 red cells were studied and the presence of positive cells was recorded.

Case reports

The clinical picture of the two older boys and a sister affected with the disease was practically identical. Tables I and II summarize the haematological and haemoglobin studies in the four patients.

III.1. The oldest child in the family, aged 11 years, was admitted to the hospital for the first time on 13 July 1964 because of fever and generalized weakness. He had been noted to be pale and had intermittent jaundice since infancy. Physical examination at the time of admission revealed mild scleral icterus and moderately severe anaemia. His head was large but the typical mongoloid facies was not apparent. The abdomen was markedly protuberant with a large spleen and a moderately enlarged liver.

Initial haematological studies revealed a moderate degree of hypochromic anaemia with normal leucocyte and platelet counts. The peripheral blood smear showed anisopoikilocytosis, many target cells, and a few nucleated red cells (Fig. 1). The reticulocyte count was elevated. Red cell osmotic fragility was decreased. Bone marrow examination was suggestive of erythroid hyperplasia.

The biochemical investigations were characteristic. Both fetal haemoglobin and the A₂ fraction were normal.

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[†] Deceased on 25 August 1973.

TABLE I
HAEMATOLOGICAL DATA OF THE FOUR SIBS WITH
HAEMOGLOBIN H DISEASE

	III.1	III.2	III.4	III.6
Age (yr)	11	9	*	3
Sex	M	M	F	M
Haemoglobin (g/100 ml)	8.3	9.5	7.5	9.8
RBC ($10^9/\text{mm}^3$)	4.60	4.30	4.00	5.12
Hematocrit (%)	32	33	35	33
MCV (μ^3)	69.0	76.7	87.5	64.4
MCH (pg)	18.0	22.1	18.7	19.1
Reticulocyte (%)	1	2.1	14	1.6
Saline fragility (% lysis in 0.4%)	13.8	10.3	30.9	6.1
Blood smear				
Macrocytosis	++	++	++	-
Hypochromia	++++	+++	++++	+
Aniso-poikilocytosis	++	++	+++	-
Target cells	++	+	++	-
Normoblasts	+	+	++	-
RBC inclusions (%)	50	10	80	15

* Deceased at the age of 1½ years.

TABLE II
BIOCHEMICAL DATA AND HAEMOGLOBIN STUDIES

	III.1	III.2	III.4	III.6
Serum bilirubin (mg %)	0.32	0.52	0.26	0.35
Serum iron (μg %)	190	163	136	—
Latent iron binding capacity (μg %)	92	230	92	—
Fetal haemoglobin (%)	1.1	0.3	2.1	0.1
A2 haemoglobin (%)	2.3	2.7	2.2	3.2
H haemoglobin (%)	19.7	2.7	30	0.3

On the other hand, starch gel electrophoresis showed in addition to haemoglobin A a definite band which at pH 8.2 had a higher anodic mobility than adult haemoglobin (Fig. 2). This migration was even more rapid at pH 6.5. The abnormal band constituted 19.7% of the total haemoglobin and was identified as haemoglobin H. This was further confirmed by the formation of multiple spherical inclusion bodies in approximately 50% of red cells.

III.2. The second male sib in the family, 9 years old, presented the same clinical picture as his brother but in a milder form. He was pale and subicteric, had a large head and a protuberant abdomen with hepatosplenomegaly. The haematological findings were similar, with the characteristic morphological changes in red cells. Again in this case haemoglobin studies showed normal levels of Hb-F and Hb-A2. Here, also the abnormal band appeared in the electrophoretic strip (Fig. 2), and

FIG. 1. Peripheral blood film from III.1. Note the hypochromia, anisocytosis, poikilocytosis, and target cells.

FIG. 2. Starch gel electrophoretic patterns of hemolysates from the family (pH 8.2). The pattern of III.4 is not shown.

typical erythrocyte were identified in approximately 10% of red cells.

III.4. The third sib, the only female, was 17 months old when first seen in the paediatric outpatient department. She had been suffering from loss of weight, fever, and repeated upper respiratory infection. On examination she appeared to be irritable, anaemic, and had stunted growth. The abdomen was greatly distended with a 4 cm enlarged spleen and 1 cm enlarged liver. The haematological changes were much more marked (Table I). This case showed the same haemoglobin findings as her elder brother (III.1), who had a wide abnormal band identified as Hb-H (Fig. 2). Incubation of her blood with BCB revealed inclusion bodies in 80–100% of red cells (Fig. 3). She died a few months later.

III.6. The youngest sib, a 3-year-old, had a negative past medical history. On examination he was found to be slightly pale but was not jaundiced; the liver and the spleen were not palpable. The haematological abnormalities were less pronounced than in the three other sibs. Red blood cell inclusions were present in about 15% of cells, and a very narrow band of Hb-H was seen on electrophoresis (Fig. 2).

Family studies

It was not possible to study all family members for the reasons mentioned above. The remaining sibs, III.3 and III.5, aged 8 and 4 years, respectively were found to be asymptomatic and their physical examination negative. There were no abnormalities in their haemoglobin patterns. However, in III.5, inclusion bodies could be detected in approximately 1/1000 red blood cells, and in both sibs the peripheral smear showed slight hypochromia, the mean corpuscular haemoglobin was low and the red cell osmotic fragility decreased. Similar findings

were noted in the mother. The other available members of the family were normal. The pedigree of the family is shown in Fig. 4.

Discussion

In three of the patients reported here the clinical and the routine haematological findings suggested the diagnosis of thalassaemia major, although in this latter condition the symptoms are usually more severe, the morphological changes in red cells more marked, and the outcome more ominous.

The diagnosis of haemoglobin H disease was based on the demonstration of intra-erythrocytic inclusion bodies and on the abnormalities of the haemoglobin pattern. The inclusion bodies were spherical, greenish blue in appearance and could be easily differentiated from the darkly-stained and filamentous structure of the reticulocyte. The abnormal band in the electrophoretic strip identified as haemoglobin H had a higher anodic mobility at pH 8.2 than normal haemoglobin. It constituted 0.3–30% of the total haemoglobin. Although the abnormal haemoglobin was not detectable in either parent of the propositi, the mother had rare inclusion bodies and a peripheral blood picture suggestive of α -thalassaemia trait. In addition, she had elevation in the Hb-A₂ fraction, consistent with heterozygosity for β -thalassaemia. The coexistence of the second condition in this family is evidenced by the severity of the anaemia in III.2 despite a very low Hb-H and the small number of inclusions.

Haemoglobin H has been shown to consist entirely of beta-polypeptide chains (Jones *et al*, 1959) with a molecular formula of β_4 . Polymerization of

FIG. 3. Inclusion bodies in red cells from III.4.

TABLE III
FAMILY STUDIES

Case	Age (yr)	Sex	RBC (10 ⁹ /mm ³)	Hb (g %)	PCV (%)	MCH (μg)	MCV (μ ³)	Reticulo- cyte Count (%)	Fragility (% lysis)	Peripheral Blood Smear	Inclusion Bodies (%)	Haemoglobin (%)		
												F	A ₂	H
III.3 (brother)	8	M	5.22	13.0	42	24.9	80.5	0.4	38.2	Normal smear	0	0.5	3.5	0
III.5 (brother)	4	M	5.03	10.6	34	20.0	67.6	0.3	10.9	Slight poikilo- and anisocytosis; mild hypochromia; no target cells	0.1	0.4	2.1	0
II.4 (mother)	29	F	4.9	11.9	36	24.5	73.5	0.4	52	Moderate anisocytosis; poikilocytosis; few target cells	Very rare	0.2	7.2	0
II.1 (father)	33	M	6.1	15.0	46	24.6	75.4	0.1	84	Normal smear	0	0.1	2.1	0
Paternal uncle	14	M	—	13.0	39	—	—	0.2	67	Normal smear	0	1.1	2.2	0
Paternal aunt	19	F	4.7	13.8	40	29.3	85.1	0.9	94	Normal smear	0	1.4	2.5	0
Paternal grand- mother	45	F	—	14.2	42	—	—	0.1	84	Normal smear	0	1.5	2.9	0

FIG. 4. Family pedigree.

the unconjugated beta chains to a tetrameric form is a direct result of the suppression of alpha-chain synthesis. This explains the presence of normal levels of Hb-F and Hb-A₂, as can be seen in our patients.

The pattern of inheritance of haemoglobin H disease has been discussed by several investigators (Motulsky, 1956; Huehns and Shooter, 1965; Weatherall, 1965). Wasi, Na-Nakorn, and Suingdumrong (1964) suggested that the disorder resulted from the interaction of two α -thalassaemia genes: one transmitted from the parent with the stigmata of thalassaemia (α_1 -thalassaemia) and a second gene, previously termed 'silent', which resulted in a milder form of α -thalassaemia (α_2 -thalassaemia). The two genes are probably allelic. In adult life it is very difficult to define these two forms of α -thalassaemia. However, in the neonatal period, α -thalassaemia is characterized by a level of

haemoglobin Barts in the range of 5–6%, an abnormal red cell morphology, and a decreased red cell osmotic fragility. On the other hand, the level of haemoglobin Barts in α_2 -thalassaemia is only 1–2%, and no morphological abnormalities can be detected in red cells (Na-Nakorn *et al.*, 1959).

Lehmann and Huntsman (1966) emphasized that in the presence of iron deficiency, the appearance of haemoglobin H may be masked due to the decrease in haem synthesis.

The presence of other fast-moving haemoglobins was ruled out because the unknown band migrated as anion both in acid and alkaline media.

REFERENCES

- Dacie, J. V. and Lewis, S. M. (eds) (1963). *Practical Haematology*, 3rd edition. Grune and Stratton, New York.
- Goldberg, C. A. J. (1958). A new method for starch gel electrophoresis of human hemoglobins, with special reference to the determination of hemoglobin A₂. *Clinical Chemistry*, **4**, 484–495.
- Gouttas, A., Fessas, P., Tseveris, H., and Xefteri, E. (1955). Description d'une nouvelle variété d'anémie hémolytique congénitale; étude hématologique, électrophorétique et génétique. *Sang; Biologie et Pathologie*, **26**, 911–919.
- Huehns, E. R. and Shooter, E. M. (1965). Human haemoglobins. *Journal of Medical Genetics*, **2**, 48–90.
- Jones, R. T., Schroeder, W. A., Balog, J. E., and Vinograd, J. R. (1959). Cross structure of hemoglobin H. *Journal of the American Chemical Society*, **81**, 3161.
- Lehmann, H. and Huntsman, R. G. (1966). *Man's Haemoglobins*, p. 176. North-Holland, Amsterdam.
- Motulsky, A. G. (1956). Genetic and haematological significance of haemoglobin H. *Nature*, **178**, 1055–1056.
- Na-Nakorn, S., Wasi, P., Pornpatkul, M., and Postrakul, S. (1969). Further evidence for a genetic basis of haemoglobin H disease from new-born offsprings of patients. *Nature*, **223**, 59–60.
- Rigas, D. A., Koler, R. D., and Osgood, E. E. (1955). New hemoglobin possessing a higher electrophoretic mobility than normal adult hemoglobin. *Science*, **121**, 372.
- Singer, K., Chernoff, A. I., and Singer, L. (1951). Studies on abnormal hemoglobins. I. Their demonstration in sickle-cell anemia and other hematologic disorders by means of alkali denaturation. *Blood*, **6**, 413–428.
- Wasi, P., Na-Nakorn, S., and Suingdumrong, A. (1964). Hemoglobin H disease in Thailand: a genetic study. *Nature*, **204**, 907–908.
- Weatherall, D. J. (1965). *The Thalassaemia Syndromes*. Blackwell Scientific, Oxford.