Hydrops foetalis due to alpha-thalassaemia: A case report

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Introductory Review

THE THALASSAEMIAS ARISE through genetically-determined reduction in the rate of synthesis of the polypeptide chains required for globin, and hence haemoglobin, formation (Itano, 1957, 1965; Ingram and Stretton, 1959; Weatherall, Clegg and Naughton, 1965). The two principal groups of thalassaemias involve depression of synthesis of alpha-chains or beta-chains respectively of adult Hb–A, but considerable heterogeneity exists within these classes (see Weatherall, 1969). Heterozygous alpha-thalassaemia trait, with partial suppression of alpha-polypeptide chains, is mild in its manifestations but of widespread geographical occurrence, and tends to occur wherever the general incidence of thalassaemia is high (see Motulsky, 1964; Weatherall, 1965; Huehns, 1965). The occurrence of Hb–H disease in such populations has long been recognised (see Huehns, 1965; Weatherall, 1969), and there is some evidence that individuals with this condition show the interaction to two different alpha-thalassaemia genes, 1 and 2 (Wasi, Na-NaKorn and Suingdumrong, 1964; Huehns, 1965). The occurrence and significance of the alpha1 – and alpha2–thalassaemia genes in Thailand has been discussed extensively by Wasi et al. (1969) and Pootrakul et al. (1970).

The pure homozygous form of alpha-thalassaemia is associated with severe hydrops foetalis, anaemia and erythroblastosis foetalis, and is incompatible with life (Lie-Injo and Jo, 1960 a, b; Lie-Injo, 1962, 1964; Lie-Injo et al., 1962). Lie-Injo and colleagues first described the condition in Chinese in Indonesia, and later found it to the extent of 1.6 per 1,000 Chinese newborns and 3.4% of stillbirths, but not in Malays or Indians in Malaysia (Lopez and Lie-Injo, 1960). Besides the occurrence in the Chinese in Singapore (Wong, 1965) and Hongkong (Banwell, 1964; Todd, Lai and Braga, 1967), this form of hydrops has been reported as quite common in Northern Thais (Bhoonsri et al., 1968) and rarely in Greek Cypriots (Diamond, Cotgrove and Parker, 1965) and Filipinos (Pearson, Shanklin and Brodine, 1965). The pathological features of the hydropic infants have been extensively reported in the above papers by Lie-Injo, Lopez and Dutt (1968). The anaemia and predominance of Hb-Bart's (Hb-gamma$_4$) in the red cells of these infants is stressed in all reports (ca. 75–90%). The remaining haemoglobin appears to be characterised by a combination of gamma-chains with unique chains similar to or identical with chains of Hb-Portland, normal Hb–A, A_2 and F being absent (Weatherall, Clegg and Wong, 1970; Todd et al., 1970). The complete suppression of alpha-chain pro-

duction seems to characterise the homozygous alpha$_1$-thalassaemia state (Weatherall, Clegg and Wong, 1970). The known high affinity of Hb-Bart's for oxygen has been stressed as a probable contributory factor to the severe effects on the foetus in alpha-thalassaemic hydrops (Wasi et al., 1969). Weatherall (1969) reviews the genetic and biochemical considerations at nuclear and cytoplasmic levels in the origin of alpha-thalassaemias.

Recognition of the heterozygous alpha-thalassaemia trait condition is difficult (see Huehns, 1965; Weatherall, 1969), and it is not easy to identify matings at risk for the production of hydropic infants. Although low levels of Hb-Bart's (5–10 and 1–2% range) occur at birth, this disappears in infancy. In the adult individuals Hb–A$_2$ levels are normal or low, not raised as in many beta-thalassaemia traits and persistence of Hb–F is unusual. Traces of Hb–H, detectable by electrophoresis of the red cell haemolysate or in the red cell incubation reticulocyte preparation, are variable and inconstant. Thalassaemic red cell morphological abnormalities may be present, mild or absent and the haemoglobin level slightly reduced or normal. Demonstration of increased osmotic resistance of the red cells to saline haemolysis in addition to their morphological abnormalities is valuable in the detection of all forms of thalassaemia trait (Beaven et al., 1964; Beaven, Dixon and White, 1966; White, Lau and Beaven, 1968), and Pearson, Shanklin and Brodine (1965) utilised this to characterise the parents of a Filipino hydropic infant. In the present report, similar methods were used in investigating the parents of a hydropic infant with high proportion of Hb-Bart's in the cord red blood cells, and dying shortly after delivery.

Case Report

A. Patient

W.K.B., a 27-year-old Chinese primigravid female, who had been married for a year, was admitted as an unbooked emergency with a history of swelling of legs and passage of only small amounts of urine for ten days and headache. She was 28 weeks gravid by dates.

On examination, she was pale and had gross ankle oedema and puffiness of face. Her blood pressure was 170/100 mm Hg. She had a grade 2/6 – cardiac systolic murmur over the praecordium. Uterine size was 34 weeks – larger than dates – and the foetus was a longitudinal lie, with a cephalic presentation in the left occipito-anterior position. Foetal heart was heard and regular. Liquor was not excessive. A diagnosis of severe pre-eclamptic toxaemia with possible hydrops foetalis was entertained.

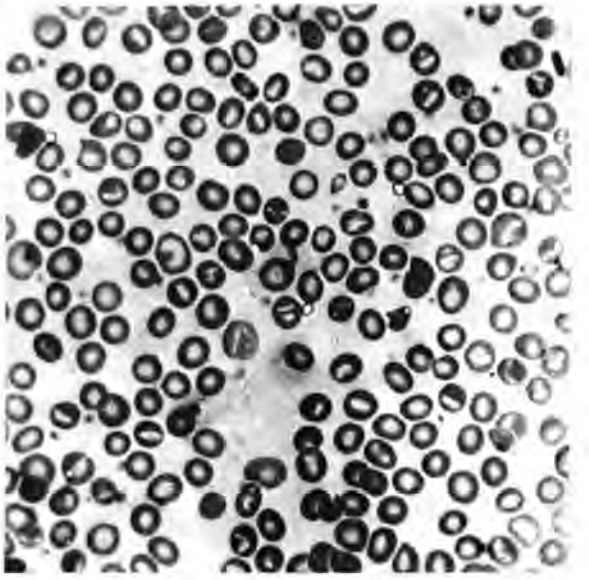

Fig. 1

Peripheral blood film of mother, showing mild red cell abnormalities. (May-Grunwald-Giemsa stain, x 600)

Investigations

Haematological investigations were by standard methods (Dacie and Lewis, 1968), and methods particularly indicated for thalassaemia were also used (Beaven, et al., 1964; Beaven, Dixon, White, 1966). Haemoglobin was 8.0 g/100 ml; PCV 30 per cent; MCV 105.7 cu.u; MCHC 26.8 per cent; blood urea 23 mg/100 ml; serum electrolytes:– Na+ – 130 mEq/1., K+ – 3.8 mEq/1., C1– 106 mEq/1. Blood group O Rhesus positive; no antibodies. ESR – 46 mm/hr.

Serum assays showed a serum iron of 96 µg/100 ml (UIBC, 284, TIBC 380ug/100 ml) and a serum folate of 3.7 ng/ml, vitamin B_{12} – 1171 pg/ml. Blood film morphology showed slight anisocytosis and anisochromasia of the red cells with some microcytosis and overall hypochromasia, and occasional elliptical, small and irregular and poikilocytic cells (Fig. 1). Osmotic fragility of the red cells was markedly decreased before and after sterile incu-

bation at 37°C for 24 hours (Fig. 2), with Median Corpuscular Fragility of 0.275 (g per 100 ml of NaCl) in both instances (normal pregnant means 0.445 and 0.550 respectively, Beaven, Dixon, White 1966). Autohaemolysis normal. Haemoglobin electrophoresis (Fig. 3c) showed Hb–A with normal A_2 (2.6 per cent) and non-haem protein (1.9%); the alkali-resistant haemoglobin fraction was normal at 0.9 per cent, and no Hb–H inclusions present in the incubation reticulocyte preparation.

(b) Urine was cloudy, acid with 2 WBC/μl. No proteinuria; on culture coliform organism were grown with a viable count of 10,000 organisms/μl.

(c) X-ray abdomen – single foetus with maldeveloped upper limbs. Gestational age 34 weeks.

She was therefore considered to be a case of alpha-thalassaemia trait (heterozygous) in addition to having P.E.T. She was treated with sedatives, hypotensive and diuretics.

On 24.1.71 at 30 weeks gestation, she began spontaneous labour and delivered a hydropic female foetus weighing 1600 g, which died soon after delivery. The placenta was large (1060 g), oedamatous, pale and friable.

B. Infant

Haematological Studies

The cord red blood cells taken at delivery were of group O Rhesus positive, no antibodies present (identical with the mother); Direct Coombs' test negative. Electrophoretic examination of the infant's red cell haemolysate showed that Hb Bart's was the major haemoglobin present (Fig. 3b, e).

The Hb-Bart's amounted to 74 per cent of the total haemoglobin. Most of the remaining haemoglobin gave a discreet band in a position similar to Hb–A, using zone electrophoresis in a discontinuous pH 8.9 tris-EDTA-borate, pH 8.6 barbital buffer system on cellulose acetate (but see recent papers by Weatherall, Clegg and Wong Hock Boon, 1970 and Todd et al., 1970 for information on the non-alpha$_2$ beta$_2$ nature of this fraction in alpha-thalassaemic hydrops). Mere traces of non-haem protein in the usual position and of a fast Hb (?Hb–H) were also present.

Alkali-resistant Hb formed 80 per cent by the ¾ minute denaturation method (Beaven, Ellis and White, 1960–61), but by determination of the rate of alkaline-denaturation, this fraction had curvilinear and faster denaturation rate than normal Hb–F, as

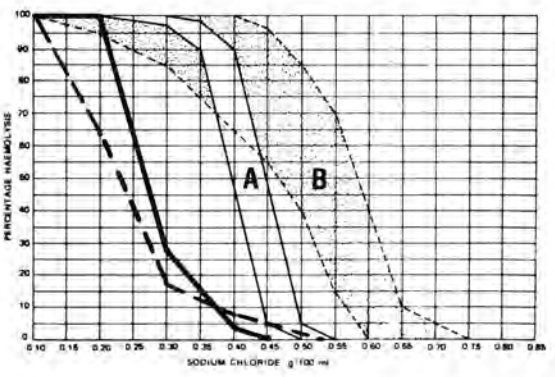

Fig. 2

Osmotic fragility of red cells of mother

—— before incubation.

— — after sterile incubation at 37°C for 24 hrs.

A normal range before incubation.

B normal range after incubation.

Fig. 3

Cellulose acetate electrophoresis at pH 8.6 of haemolysates of family with alpha-thalassaemia

a. Hb-H disease control.
b. Hydropic infant.
c. Mother of hydropic infant.
d. Father of hydropic infant.
e. Hydropic infant.
f. Normal cord blood control
*** NHP, non-haem protein.**
**** Hb fraction in hydropic infant haemolysate in position of Hb–A under these conditions.**

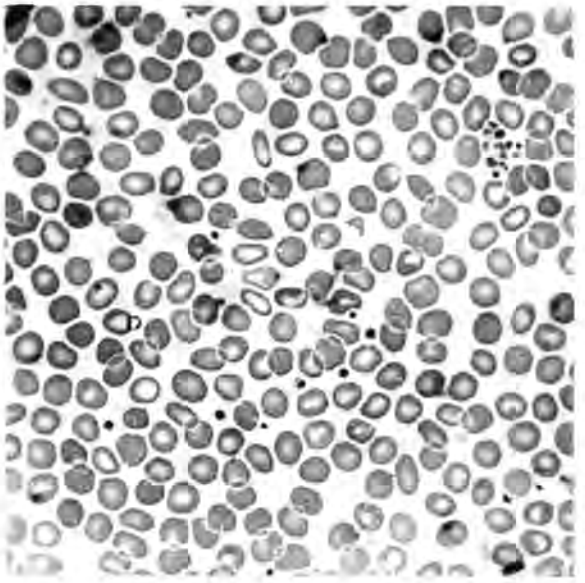

Fig. 4

Peripheral blood of father, showing mild red cell abnormalities and occasional elliptical cells. May-Grunwald-Giemsa stain, x 600.

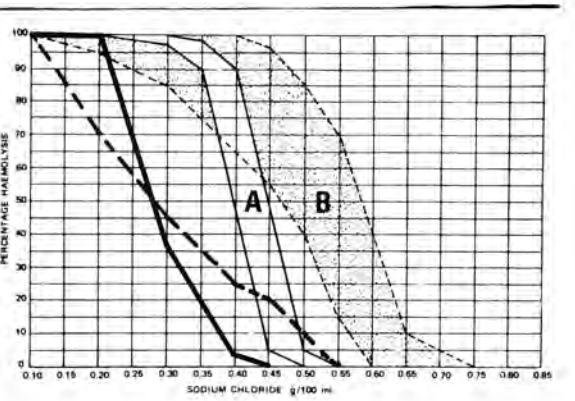

Fig. 5

Osmotic fragility of red cells of father:—

——— before incubation.

— — after sterile incubation at 37° C for 24 hrs.

A normal range before incubation.

B normal range after incubation.

found by Ager and Lehmann (1958) for Hb-Bart's.

Post-Mortem Examination

Revealed ascites, an enlarged congested liver and spleen, with numerous foci of erythropoiesis in both. The cause of death was considered to be hydrops foetalis and erythroblastosis.

Puerperium

Routine abdominal examination revealed bilateral cystic masses in both iliac fossae which, on laparotomy, were confirmed to be bilateral ovarian serous cystomas. Bilateral ovarian cystectomy was carried out.

Since discharge and at post-natal follow-up, she has remained well.

Haematological examination four months after delivery showed Hb 12.2 g/100 ml, PCV 42, MCHC 29 per cent. Red cell characteristics as before; no Hb—H inclusion bodies detected, and red cells containing Hb—F 0.2 per cent (more than might be expected). Electrophoretic composition of the haemolysate unchanged. Serum assays have shown iron varying from 52 µg/100 ml post-delivery to 114 µg/100 ml 2½ months later. Folate levels were satisfactory (18.8 ng/ml) two months after a course of oral folic acid therapy.

C. Haematological Examination of Husband

The husband's blood was examined after the wife's delivery. The haemoglobin was 14.5 g/100 ml, PCV 45, MCHC 32.2 per cent. The blood film showed mild morphological abnormalities, with slight anisocytosis, anisochromasia, hypochromasia and occasional elliptical and elongated cells (Fig. 4). The osmotic resistance of the red cells was markedly increased with MCF of 0.270 before incubation and 0.230 (g/100 ml of NaCl) after incubation (Fig. 5). Autohaemolysis normal. Serum assays, iron 126 µg/100 ml (UIBC, 141, TIBC 267 µg/100 ml).

Haemoglobin electrophoresis showed Hb—A as the major component (Fig. 3d), with normal Hb—A_2 (2.1 per cent) and non-haem protein (1.4 per cent); alkali-resistant Hb normal at 0.8 per cent, and no Hb—H inclusions detected in the incubation reticulocyte preparation.

The findings were considered compatible with the

presence of the alpha-thalassaemia trait. As both parents carry the same abnormal trait, it is possible for the hydropic infant to be homozygous for this condition.

Discussion

Clinical and Pathological Considerations

Hydrops foetalis can result from a number of causes, e.g. haemolytic disease associated with (1) Rhesus or less commonly ABO group incompatibility (see Mollison, 1967; Weiner, 1968), (2) congenital syphilis, (3) severe congenital heart disease (Donald, 1969), (4) homozygous alpha-thalassaemia. In much of S.E. Asia, and in Malaysia particularly, Rh incompatibility is rare apart from the Indian population, though ABO incompatibility is not uncommon. Amongst the Chinese particularly, the occurrence of homozygous alpha-thalassaemia must be considered. The finding of a very large proportion of Hb-Bart's (Hb – gamma$_4$) in the red cells of infants is highly suggestive. Such infants are usually born dead at 28–34 weeks, or die shortly after delivery, and have generalised hydrops, ascites and hepatosplenomegaly with erythroblastaemic anaemia and marked extramedullary haemopoiesis. Further confirmation is obtained if the heterozygous alpha-thalassaemia trait can be demonstrated in the blood of both parents (see Weatherall, 1965, and references in Introductory Review).

The finding of Hb-Bart's in the infant's red cells is not unique to this form of hydrops; it is the very high proportion which is significant (ca. 75–90%). Smaller amounts are common in cord blood cells, particularly in populations where thalassaemia is common (Hendrickse, 1965; Weatherall, 1965; Beaven, Dixon and White, 1966), but the proportions are far lower (<1–2% and 5–10%), and disappear together with the decline in Hb–F during the first six months of life (Ager and Lehmann, 1958). Some such infants will subsequently be found to be healthy but carry the alpha-thalassaemia trait, whilst in others a higher proportion of Hb-Bart's is replaced wholly or partly by Hb–H in the presence of Hb–H disease (see Huehns, 1965; Weatherall, 1969).

The identification of the mother-at-risk in advance and her expectant management depends upon recognition of the alpha-thalassaemia trait. The generally agreed difficulty in doing this has been commented upon above. The general characteristics of the thalassaemia-trait blood picture are (Beaven, Dixon and White, 1966):–

(a) abnormalities of red cells, including microcytosis.
(b) reduced mean corpuscular haemoglobin concentration (MCHC).
(c) reduced osmotic fragility of the red cells before and after incubation.
(d) presence of iron (often increased) in storage reticulum cells and erythroblasts of the bone marrow.
(e) significant proportions of foetal haemoglobin.
(f) marginally or significantly elevated Hb–A_2 levels.

Abnormalities (e) and (f) apply particular to the beta-thalassaemia traits, and not to the alpha-type.

Both alpha- and beta-thalassaemia traits are not uncommon in Malaysia. Morphological changes in the red cells are of very frequent occurrence in Asian populations, and are often attributable to nutritional deficiencies, particularly iron deficiency. Even where this can be excluded with the help of serum assays, etc., the abnormalities are not always readily assignable to any definite genetic trait (White, Lau and Beaven, 1968). In the alpha-thalassaemia trait, the haemoglobin level may be normal or only slightly reduced, Hb–A_2 levels are not raised as is common in the beta-trait, Hb–F is not usually raised and presence of traces of Hb–H is inconstant. The determination of red cell osmotic fragility before and after incubation of the cells is valuable in that the resistance is often markedly increased, but the test is time consuming. Danon (1963) has described a rapid micro method for recording red cell osmotic fragility by continuous decrease of salt concentration which could extend the scope of this investigation, though requiring special apparatus.

Genetic Counselling

It will usually come about that hydrops foetalis due to homozygous alpha-thalassaemia is detected only after birth of an affected child, which may be a first child as in the case reported. The wider question of recognising matings-at-risk in advance depends upon widespread application of techniques for the often difficult detection of the alpha-trait state in the parents-to-be.

In either event, the Mendelian segregation of the abnormal gene indicates that for every severely affected child, two children with the same mild heterozygous trait as the parents may be expected to be born,

and one completely free of the trait. Nonetheless, although this is the expected overall distribution, the order is not necessarily conformed to, and repeated pregnancies may result in further affected infants, as in the three successive cases described by Banwell and Strickland (1964).

Summary

1. A case of hydrops foetalis is described in a female Chinese infant dying shortly after spontaneous birth at 30 weeks of gestation. There was severe anaemia (3.6 g/100 ml), and the red cell haemolysate contained ca. 74 per cent of Hb-Bart's.
2. The alpha-thalassaemia trait was recognised in both parents by the occurrence of minor red cell abnormalities but markedly increased osmotic resistance in the presence of normal proportions of Hb–A, Hb–A_2 and Hb–F in the haemolysate; there was no reduction in serum iron levels.
3. The clinical, pathological and genetic features of the homozygous condition are discussed, and also the problem of recognising the mild, heterozygous alpha-thalassaemia trait.
4. The importance is stressed of recognising homozygous alpha-thalassaemia as a cause of hydrops foetalis in S.E. Asia, among Chinese populations particularly. The parents may be advised that normal and minimally-affected trait carriers may also be expected among their progeny.

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