HAEMOGLOBIN E SYNDROMES IN A CEYLONESE FAMILY

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Haemoglobin E is an abnormal type of human haemoglobin which appears to be the prototype in South-east Asia of sickle-cell haemoglobin in Africa. It was first reported in a North American child of partial Asiatic extraction (Itano, Bergren, and Sturgeon, 1954), and has since been shown to occur in Burma, Thailand, Malaya, Indonesia, Sarawak, Cambodia, and Bengal; single instances have been reported in a Persian and an Eti-Turk. In Ceylon it was first reported in the aboriginal Veddahs (Graff, Ikin, Lehmann, Mourant, Parkin, and Wickremasinghe, 1955), and further studies have shown that haemoglobin E also occurs in the general population, and it has been the only abnormal haemoglobin so far encountered in the island (de Silva, Jonxis, and Wickremasinghe, 1957).

The present paper is a report on a Sinhalese* family in Ceylon consisting of the parents and five children. The father carried the haemoglobin E trait, the mother was considered to have carried the thalassaemia trait, three of the children were found to be affected with thalassaemia-haemoglobin E disease, and two children were normal.

Methods and Materials

All blood counts were made on venous blood samples collected in Wintrobe mixture. Erythrocytes and platelets were counted, using pipettes, counting chambers, and coverslips conforming to A.H.T. Co. specifications. Haemoglobin was determined by the cyanhaematin method (Dacie, 1956). Haematocrit measurements were made in Wintrobe tubes after centrifugation at 3,000 revolutions a minute for 30 minutes. Reticulocytes were counted on fixed alcoholic brilliant cresyl blue preparations counterstained with Leishman's stain. Schumm's test, Coombs's test, and the staining for Heinz bodies and siderocytes were performed as described by Dacie (1956). Tests for sickling were done with sodium metabisulphite. Quantitative osmotic fragility was

determined by the method of Dacie and Vaughan (Dacie, 1956), and serum bilirubin estimated as described by King (1946).

Haemoglobin types were identified by paper electrophoresis in 0.05 M barbitone buffer at pH 8.6, as well as in 0.05 M phosphate buffer at pH 6.5. Haemoglobin F was estimated by the alkali-denaturation test described by Singer, Chernoff, and Singer (1951). For confirmation of the haemoglobin types, blood samples were sent to Professor J. H. P. Jonxis, Department of Paediatrics, University of Groningen, The Netherlands.

Case 1

G.T., a Sinhalese girl, aged 13, was admitted to the General Hospital, Colombo, in February, 1957, for continued fever of three months' duration, abdominal swelling, and marked dyspnoea. Her previous history revealed that she had suffered from shortness of breath and an abdominal swelling with distress in the left upper quadrant from the age of 2 years. She had been treated several times at her local hospital, as these symptoms were progressive. During this time she had also suffered from several bouts of fever with chills, rigors, vomiting, diarrhoea, and jaundice. Each attack lasted for about a week and recurred at intervals of two to three months.

On examination the patient was ill-nourished, apathetic, and extremely pale. Her temperature was 100.6° F. (38.1° C.), her height 4 ft. 2 in. (127 cm.), and her weight 54 lb. (24.5 kg.). She had a protuberant abdomen, wasted extremities, and a dry wrinkled skin. The conjunctivae were not icteric. There were no leg ulcers and the lymph nodes were not palpable. There was mild oedema of the ankles. The heart was enlarged and a soft grade II systolic murmur was heard at the apex. The liver was palpable three fingerbreadths below the right costal margin, was slightly tender and moderately firm, and had a smooth surface. The spleen was greatly enlarged, and extended 1 in. (2.5 cm.) below the umbilicus. It had a sharp edge, a smooth surface, and was not tender.

Laboratory Findings.—The urine showed a trace of albumin and bile salts, there was no sugar, and urobilin was detectable at a 1/20 dilution. Serum:—Total serum proteins were 6.4 g./100 ml. (albumin 2.37 g., globulin 3.72 g., fibrinogen 0.34 g.); serum bilirubin was 1.8 mg. per 100 ml.; icterus index 11 units; van den Bergh reaction direct delayed; Takata-Ara ++. Blood:—The findings are for the most part summarized in the Table; other findings were white blood cells 7,200 per c.mm., (68% polymorphonuclears, 32% lymphocytes); the blood picture showed anisocytosis, poikilocytosis, polychromasia, basophilic stippling, and hypochromia. Two attempts at marrow aspiration (sternum and iliac) were unsuccessful.

X-ray examination showed an enlargement of the heart, congestion of the lungs, and no abnormality of the skull and tubular bones; the hands showed rarefaction.

Haemoglobin:—No alkali-resistant fraction was detected, and paper electrophoresis revealed the presence of two components—one with the mobility of normal adult haemoglobin and the other with that of haemoglobin E.

Haematological Data

Case	Diagnosis	R.B.C. (mil./ c.mm.)	Haemo- globin (g./ 100 ml.)	P.C.V. (%)	M.C.V. (μ ³)	M.C.H.C. (%)	Reticu- lyocytes (%)	Platelets	Nucle- ated R.B.C.	Target Cells	Fragility
1. G.T.	Thalassaemia-haemoglobin E disease	0.85	2.1	8.5	100	25	17.2	42,000	0	0	0.38-0.32
2. C.T.	" " " "	2.93	6.6	23.0	78	29	2.8	162,000	+	++	0.38-0.18
3. P.R.T.	Normal " " " "	4.22	11.1	35.0	83	33	0.5	76,000	0	0	0.50-0.26
4. B.M.T.	" " " "	4.08	11.4	35.0	86	33	1.0	14,000	0	0	0.40-0.28
5. A.T.	Thalassaemia-haemoglobin E disease	3.50	6.9	31.0	89	22	4	12,100	+	+++	0.44-0.16
6. Mr. T.	Haemoglobin E trait	—	—	—	—	—	—	—	0	0	—
7. Mrs. T.	Thalassaemia trait	—	—	—	—	—	—	—	0	0	Cells resistant to haemolysis in distilled water

None of the blood samples showed sickling, malarial parasites, Heinz bodies, or siderocytes; Coombs's and Schumm's tests were negative in all cases.

*The Sinhalese are the majority community in Ceylon.

Thalassaemia-haemoglobin E disease was diagnosed (see Discussion).

The patient had a low fever throughout her illness with an occasional rise in temperature accompanied by chills and rigors; the highest temperature recorded was 102° F. (38.9° C.). She had 12 blood transfusions—each of 250 ml. at weekly intervals—and was treated with penicillin for the fever. Her condition deteriorated, there being only a slight response to the transfusions, and she died three months after admission. The cause of death was thought to be congestive heart failure and an aplastic crisis due to congenital haemolytic anaemia. A necropsy was not performed.

Case 2

C. T., aged 10, brother of Case 1, with a history similar to hers, was admitted to hospital for an abdominal swelling of several years' duration.

On examination his height was 3 ft. 1½ in. (95 cm.) and his weight 48 lb. (21.8 kg.). He was pale, with a yellowish coloration of the skin. The conjunctivae were icteric and the cheek-bones high. The abdomen was protuberant. There were no active leg ulcers, but old scars were evident. Lymph nodes were not palpable. The heart was not enlarged and there were no murmurs. The liver edge was palpable three fingerbreadths below the right costal margin, and was firm but not tender. The spleen was grossly enlarged down to the level of the iliac crest; its surface was smooth, and it was not tender.

Laboratory Findings.—Urine:—Urobilin was present; there was no albumin, bile, or bile pigments. Serum:—Serum bilirubin was 6.8 mg. per 100 ml.; van den Bergh reaction, direct delayed; Takata-Ara ++; and the cephalin-cholesterol flocculation test +++. Blood:—The findings are for the most part summarized in the Table; other findings were: white blood cells, 4,200 per c.mm. (46% polymorphonuclears, 45% lymphocytes, 9% eosinophils); the blood picture showed anisocytosis, poikilocytosis, perry forms, and microcytosis; there were many target cells and an occasional nucleated red cell (Fig. 1).

X-ray examination revealed trabeculations, which were most marked in the metacarpal bones, phalanges, and lower ends of the tubular bones; there were no changes in the skull.

Haemoglobin:—Alkali-resistant haemoglobin was significantly raised (5%), and paper electrophoresis revealed the presence of haemoglobin E and haemoglobin F.

On the above findings a diagnosis of thalassaemia-haemoglobin E disease was made. The patient was discharged from hospital and is attending school.

Family Study

Case 3.—P. R. T., a boy aged 7, the third in the family, had no history of anaemia, jaundice, chills, or rigors. Physical examination and laboratory studies revealed no abnormalities. The blood findings are summarized in the Table. The haemoglobin was normal.

Case 4.—B. M. T., a girl aged 6, the fourth in family, was similar to Case 3.

Case 5.—A. T., a girl aged 4, the youngest in the family, had no history of definite illness, but presented suggestive signs. She was pale and her conjunctivae were icteric. The heart was not enlarged, the liver was palpable 1½ fingerbreadths below the right costal margin, and the spleen was not palpable. The blood findings are summarized in the Table. X-ray examination showed trabeculations in the metacarpals and phalanges of both hands. Haemoglobin:—Alkali-resistant haemoglobin was significantly increased and paper electrophoresis showed haemoglobins E, A, and F. Thalassaemia-haemoglobin E disease was diagnosed.

Case 6.—Mr. T., aged 40, the father, had no symptoms of a haemoglobinopathy, and physical examination showed no abnormalities. Haemoglobin:—No alkali-resistant haemoglobin was present, but paper electrophoresis revealed the presence of haemoglobins E and A. He was regarded as an asymptomatic carrier of haemoglobin E trait.

Case 7.—Mrs. T., aged 40, the mother, gave a history which revealed no untoward features. The blood picture was not unusual, but her red blood cells were found to be resistant to lysis in distilled water. Haemoglobin:—No alkali-resistant or abnormal haemoglobins were present, but she is considered to be a carrier of the thalassaemia trait (see Discussion).

Discussion

No studies have been made of the relative prevalence of haemoglobin A₂ and haemoglobin E among the Ceylonese. This is unfortunate, since these haemoglobins are indistinguishable by paper electrophoresis in barbitone buffer at pH 8.6 or phosphate buffer at pH 6.5. However, quantitative measurements indicated that haemoglobin A₂ constituted 1.8 to 3.5% of the total haemoglobin in a series of 26 individuals studied by Kunkel and Wallenius (1955). In the cases described here, the amount of the slow-moving haemoglobin component was at a rough estimate much higher than this figure, and was accordingly identified as haemoglobin E.

Case 1 presented unusual features. Paper electrophoresis and a negative alkali-denaturation test indicated that she carried the haemoglobin E trait; she was severely affected and ultimately died. These observations are contrary to those of Chernoff, Minnich, Na-Nakorn, Tuchinda, Kashemsant, and Chernoff (1956), that haemoglobin E in the trait form is associated with an entirely benign asymptomatic carrier state. In their series of 30 individuals with haemoglobin E trait, who were apparently free from other incidental diseases, there was no consistent history of anaemia, cardio-respiratory distress, jaundice, leg ulcers, or arthralgias, and the haematological findings were not unusual. However, in our Case 1, although the haematological findings, except for the evidence of severe anaemia, were normal, the patient had been physically incapacitated from the age of 2 years with progressive anaemia, attacks of jaundice, chills, fever, shortness of breath, and hepatosplenomegaly. Hence, although haemoglobin F was not detectable by the alkali-denaturation test, hers is most probably a case of thalassaemia-haemoglobin E disease in view of the clinical picture, the fatal anaemia, and the fact that carriers of the haemoglobin E trait are symptomless or show only mild

FIG. 1.—Stained peripheral blood films in Case 1 (left) and in Case 2 (right).

symptoms. Alternatively, she might have been a case of haemoglobin E trait (or even thalassaemia trait) with anaemia due to some entirely different cause.

Case 2 had haemoglobin E and about 5% haemoglobin F in his blood and showed signs of severe anaemia. He was evidently a case of thalassaemia-haemoglobin E disease, but the relatively low figure of 5% is in contrast to the values of 20-40% obtained in Siamese by Chernoff (quoted by Lehmann, 1957). The absence of demonstrable haemoglobin A is in keeping with the findings of other workers that the presence of the thalassaemia gene interferes with the expressivity of the gene responsible for the production of haemoglobin A.

Cases 3 and 4 were unaffected and showed only haemoglobin A in their bloods. It follows that neither of the parents of this family could be homozygous for either the haemoglobin E gene or for the gene (or genes) responsible for thalassaemia. Analysis of the parents' bloods showed the presence of haemoglobins E and A in the father, and only haemoglobin A in the mother. Hence the appearance of haemoglobin F in Cases 2 and 5 may have been due either to a mutation or to inheritance from one of the parents.

The suggestion of mutation is a purely academic one, since all indications are that mutation from a normal gene

FIG. 2.—Genetic representation of the family.

to abnormal at any given locus takes place only once in 100,000 gametes, so that it is only in cases like those of the ABO and MN groups, where most members of families have been tested, or in congenital diseases like haemophilia, where the resulting disease has striking manifestations, that instances of human mutation may be expected to be found. Moreover, the presence of two separate identical mutations in a single sibship is extremely unlikely and would occur in only one in 10,000,000,000 families for each gene. Hence one of the parents in the family studied must have been a carrier of the thalassaemia gene. It is perhaps reasonable to infer that the gene is present in the mother, because had it been present in the father he would have shown at least some of the rather severe manifestations of thalassaemia-haemoglobin E disease. We therefore regard the mother as a carrier of the thalassaemia trait with non-penetrance of the thalassaemia gene: that she was not entirely normal was indicated by the increased resistance of her red blood cells to haemolysis. Accordingly we have constructed the genetic table as illustrated in Fig. 2.

A possible racial origin of haemoglobin E cannot be ascertained as yet. This abnormal haemoglobin probably arose in South-east Asia as the result of a single mutation and was then disseminated from this focus. With regard to Ceylon, the Sinhalese as well as the Veddahs are of partial Bengali extraction, and it is possible that the haemoglobin E found in Veddahs and Sinhalese originated in Bengal.

Mackey and Vivarelli (1954) suggested that sicklers are more prone to develop falciparum malaria than non-sicklers. Allison (1954) and Raper (1955) reported statistically significant differences in the incidence of malaria in carriers of the sickle-cell trait and those without the trait. Others (Moore, Brass, and Foy, 1954; Archibald and Bruce-Chwatt, 1955) could not confirm the differences. No statistical investigation has been done so far to determine

whether carriers of haemoglobin E were more resistant to malaria than those without haemoglobin E. Na-Nakorn, Minnich, and Chernoff (1956) reported that haemoglobin E offered no protection against malaria. Still, it is interesting to note that the incidence of haemoglobin E in Ceylon is largely confined to areas which had been hyperendemic for malaria prior to 1945 (de Silva *et al.*, 1957).

Summary

Clinical, haematological, and genetic manifestations of haemoglobin E syndromes in a Ceylonese family are described. In this family, the father carried the haemoglobin E trait, three children were affected with thalassaemia-haemoglobin E disease, whilst the mother and two other children showed only normal adult haemoglobin in their bloods.

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FRAMYCETIN IN INFANTILE GASTRO-ENTERITIS DUE TO PATHOGENIC ESCHERICHIA COLI

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Framycetin sulphate ("soframycin") is an antibiotic produced by a strain of *Streptomyces* which was isolated by Decaris in 1947 (Decaris, 1953). Given parenterally, this substance is highly toxic to the kidneys and the eighth cranial nerves (Massénat-Deroche, 1954), a fact which has retarded its clinical use. When given by mouth, however, it is harmless at the prescribed dosage levels, since it is very poorly absorbed from the gut.

In vitro its action on various strains of pathogenic *E. coli* is far superior to that of chloramphenicol and the tetracyclines. It is both bacteriostatic and bactericidal (Massénat-Deroche, 1954).

Various investigators have reported on its usefulness in infective infantile gastro-enteritis (Marquézy *et al.*, 1955; Laplane *et al.*, 1955; Cathala *et al.*, 1954; Schneegans *et al.*, 1955, 1956; Boyer and Tissier, 1955; Pierret *et al.*, 1956; Shidlovsky *et al.*, 1955-6).