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Thalassaemia in Ceylon

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Thalassaemia has a widespread geographical distribution. It occurs in the Mediterranean populations but has also been found in the Middle East, India and South-East Asia and in the people of the Mediterranean stock elsewhere. It represents a genetically inherited disturbance in which haemoglobin formation is inhibited. Haemoglobin has four peptide chains, two pairs being identical. Four different chains have been found in the 3 haemoglobins F, A and A_2 and are referred to as α, β, γ and δ chains. Thus the three normal haemoglobins may be designated as $\alpha_2\beta_2$ (HbA), $\alpha_2\gamma_2$ (HbF) and $\alpha_2\delta_2$ (HbA_2). The basic mechanism of diminished haemoglobin synthesis in thalassaemia is not known. Available evidence is in favour of the hypothesis that thalassaemia is caused by an abnormality in the genetic factors which govern the rate of synthesis of α and β chains of the normal adult haemoglobin. The classical thalassaemia is considered to be caused by diminished production of β chains. Here the synthesis of α, γ and δ chains is not disturbed, hence the relative and absolute amounts of HbF and HbA_2 are increased. On the other hand, if the production of α chain is diminished that of HbF and HbA_2 are also impaired. The excess of β chain tetramerize to form HbH (β_4) an unstable haemoglobin which precipitates. The variety of thalassaemia accompanied by HbH or Bart's has been referred to as α-thalassaemia (1).

Clinically, the severity of the disorder varies considerably ranging from severe anaemia to that of an asymptomatic trait.

Methods and Materials

The methods used in the haematological investigations have been described in a previous communication by NAGARATNAM *et al.* (2). Electrophoresis of haemoglobin was carried out using paper at pH 8.6 (veronal) and 8.9 (Tris buffer). Evaluation of HbA_2

fraction was made using control samples of haemoglobin with normal and raised HbA_2. Alkali-denaturation technique of SINGER *et al.* (3) was used for estimating foetal haemoglobin. Erythrocyte inclusion bodies was demonstrated using brilliant cresyl blue vital staining.

Case Reports

α (Alpha)-Thalassaemia (Thalassaemia-HbH Disease)

Case 1 (Mudiyanse family). A female child (N7) aged 5 months was admitted with a history of breathlessness, listlessness and fever. On examination she was febrile, pale and had a yellowish discoloration of her skin. The lymph glands were not palpable. The liver was one fingerbreadth below the right costal margin. The spleen was not palpable.

Haemoglobin 2.2 g%, PVC 9%, MCV 75.0 μm^3, MCHC 25.3%, WBC 47,000 per mm^3 inclusive of nucleated red cells. DC: N: 70%, L. 25%, E 4%, M 1%. Blood film showed anisopoikilocytosis, hypochromia, nucleated red cells and target cells (Fig. 1). Inclusion bodies were seen in the erythrocytes with brilliant cresyl blue vital staining (Fig. 2). Tests for sickling negative. Serum bilirubin 1.8 mg%. Urine: bile nil, urobilin nil, X ray of hands tubular bones and skull showed no abnormality. Haemoglobin electrophoresis showed a fast moving fraction besides HbA (Fig. 3). Since the blood showed inclusion bodies in the erythrocytes this was probably HbH.

α-Thalassaemia (thalassaemia-HbH disease) was diagnosed. The child was transfused, but was removed from hospital by the parents to be admitted a few days later in a critical condition and died the following day. No further studies were possible.

Family study (parents and siblings): Clinical examination of the family revealed no abnormality. Alkali resitant Hb ranged from 2.3—4.4%, highest being in the youngest (aged 1½years) of the surviving children. No abnormal haemoglobins were detected.

β (Beta)-Thalassaemia

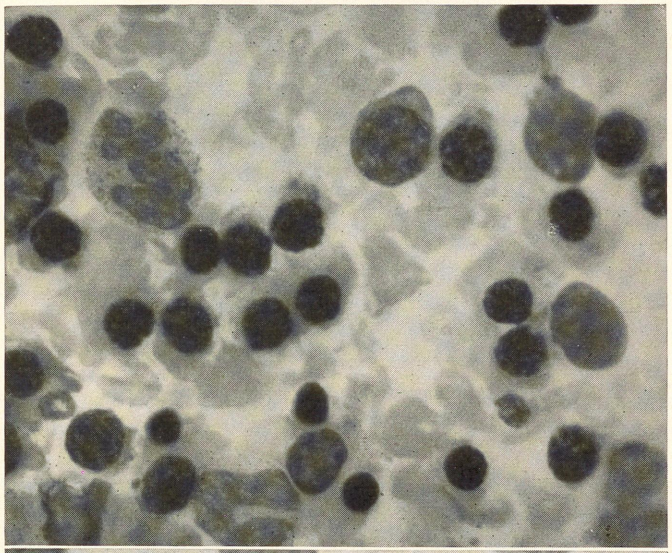
Case 2 (Martin Singho family). A male child (M5) aged 8 months was admitted with a history of swelling of his abdomen, pallor and failure to thrive all of one month's duration. On examination, the child was thin and emaciated, was febrile and extremely pale. Examination of the heart revealed a systolic murmur, haemic in type. The abdomen was distended, the liver and spleen were palpable about 3 fingerbreadths below their costal margins.

Haemoglobin 4.2 g%, PVC 17%, MCV 65.4 μm^3, MCHC 24%. The blood picture showed anisopoikilocytosis, hypochromia, target cells and a large number of nucleated red cells (Fig. 4). WBC 210,000 mm^3 inclusive of the large number of nucleated red cells. DC: P. 30%. L 44%, E 26%, Reticulocytes 6%, Total proteins 7.04 g%, Alb/glob ratio 0.2. Serum bilirubin nil, thymol turbidity 8 units, cephalin cholesterol 2+ tests for sickling were negative. X rays of the skull, hands and tubular bones showed no abnormalyity. Alkali-resistant haemoglobin was 36.4% and paper electrophoresis of the sample revealed HbA and HbF.

β-Thalassaemia (thalassaemia major) was diagnosed. The child was given a blood transfusion and since then had received two more.

Family study (parents and 4 siblings): Mother: Clinical examination revealed no obvious abnormality. The blood picture showed hypochromia, and paper electrophoresis revealed raised HbA_2 with no abnormal haemoglobins (Fig. 5). Clinical examination of the rest of the family revealed no abnormality. Alkali-resistant haemoglobin was significantly raised. No abnormal haemoglobin was detected on paper electrophoresis.

Case 3 (Punchi Banda family). A male child aged 8 months was admitted with a history of abdominal swelling of two months duration. On examination he was pale



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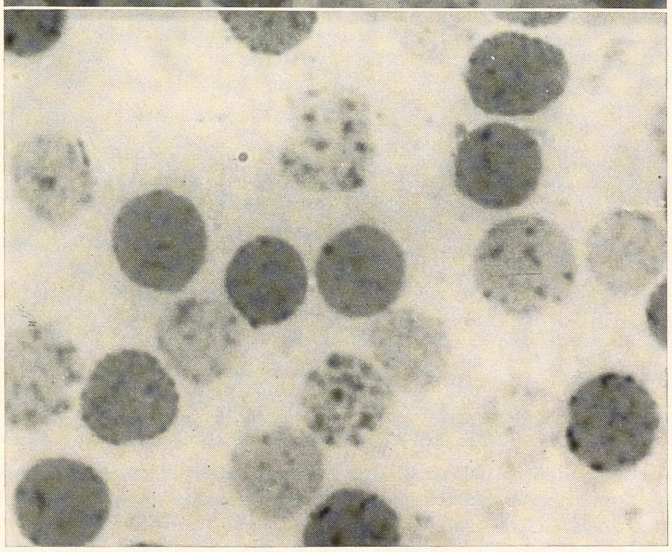

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Fig. 1. Case 1: Peripheral blood picture showing a large number of nucleated red cells in varying stages of maturation.

Fig. 2. Case 1: Erythrocyte inclusions in propositus N7. Brilliant cresyl blue vital staining. The dense dark bodies in some of the cells are reticulum stained.

and breathless. No lymph glands were palpable. The liver was palpable 2 fingerbreadths below the right costal margin and the spleen 4 fingerbreadths below the left costal margin. No other abnormality was detected.

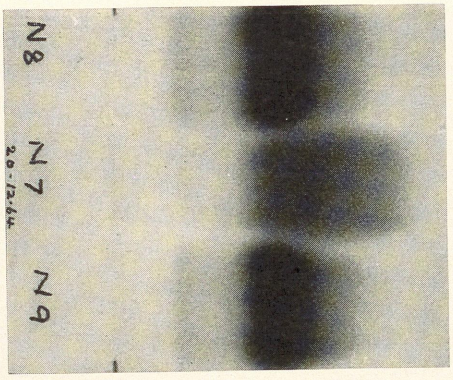

Fig. 3. Case 1: Paper electrophoresis showing fast moving fraction of Hb trailing forward in propositus N7.

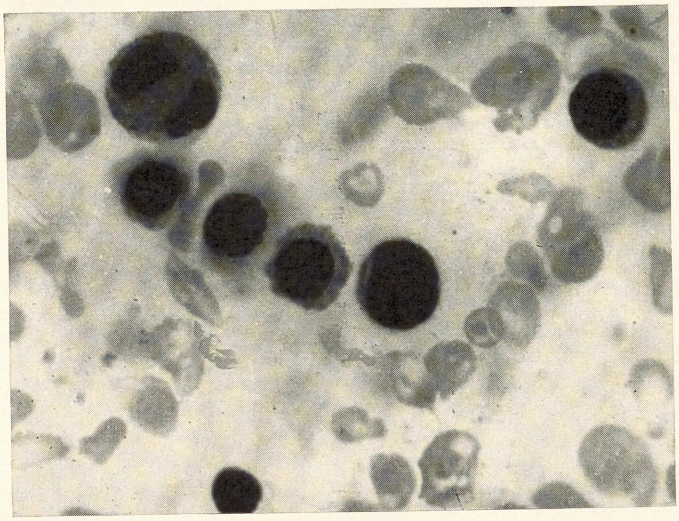

Fig. 4. Case 2: Peripheral blood picture showing anisopoikilocytosis, hypochromia, target cells and nucleated red cells.

Haemoglobin 3.3 g%, PCV 14%, MCV 77.7 μm^3, MCMC 23.5%, reticulocytes 6%, WBC 50,000 mm^3 (inclusive of nucleated red cells). DC: P 34%, L 54%, E 9%, premyelocytes 3%. The blood picture showed anisopoikilocytosis, hypochromia, a large number of normoblasts and a few target cells. White cells showed a shift to the left. Tests for sickling negative. Intraerythrocytic inclusions: negative. Serum bilirubin 0.2 mg%, thymol turbidity 4 units, cephalin cholesterol 1+, zinc sulphate turbidity 16 units. Total proteins 6.0 g%, A/G ratio 0.62. X rays revealed no abnormality. Alkali-resistant haemoglobin was found to be 73.5% and electrophoresis revealed a single component with the mobility similar to HbF.

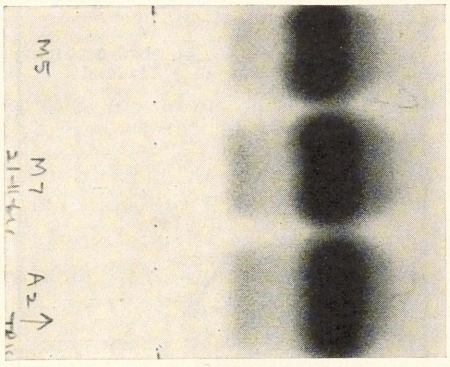

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Fig. 5. Case 2: Paper electrophoresis pH 8.9 (TRIS buffer) showing increased HbA_2 in mother compared with a control. The propositus (M5) shows HbA+F.

β-Thalassaemia (thalassaemia major) was diagnosed. The child was treated with blood transfusions.

Family study: The mother's blood showed normal amount of foetal Hb. (1.27%) and on electrophoresis no abnormal harmoglobin was detected. Haemoglobin A_2 was found to be slightly increased.

Thalassaemia with β-Chain Anamolous HbE

The following family was described earlier in detail by one of us (2) and therefore will not be discussed here in detail.

Case 4 (Tennekoon family): A girl aged 13 years was admitted with continued fever, abdominal swelling and dyspepsia. She was pale and apathetic. The liver and spleen were considerably enlarged.

Haemoglobin 2.1 g%, PCV 8.5%, MCV 15,0 μm^3, MCHC 25%, reticulocytes 17.2%, WBC 7000 mm^3. DC: P 68%, L 32%. Osmotic fragility 0.38%—0.32%. The blood picture showed anisopoikilocytosis, polychromasia and basophilic stippling and hypochromia. Serum bilirubin 1.8 mg%. X rays of the skull and tubular bones showed no abnormality, the hands showed rarefaction. No alkali-resistant haemoglobin fraction was detected and paper electrophoresis revealed two components, one with the mobility of HbA and the other of HbE. In spite of failure to detect HbF a diagnosis of thalassaemia-HbE disease was made and this had been discussed in a previous communication (2).

Family study. A brother of case 4 aged 10 years was admitted with abdominal swelling of several years duration. The child was pale the liver and spleen were grossly enlarged. Haemoglobin 6.6 g%, PCV 23%. MCV 78 μm^3, MCHC 29%, reticulocytes 2.8%, WBC 4200 mm^3. DC: P 46%, L 45%, E 9%. The blood picture showed anisopoikilocytosis, pessary forms microcytosis and many target cells and an occasional nucleated red cell. Serum bilirubin 6.8 mg%. X rays revealed trabeculations which were marked in the metacarpal bones, phalanges and lower ends of the tubular bones. No changes were seen in the skull. Alkali-resistant Hb was significantly raised (5.0%) and paper electrophoresis revealed the presence of HbE and HbF. Thalassaemia-HbE was diagnosed. One other member of the family had thalassaemia-HbE disease. The father was regarded as an asymptomatic carrier of HbE trait, and the mother a carrier of thalassaemia trait.

Case 5 (Samel family): A female child aged 6½ years was admitted with swelling of her abdomen, tiredness and loss of appetite all of two months duration. On exam-

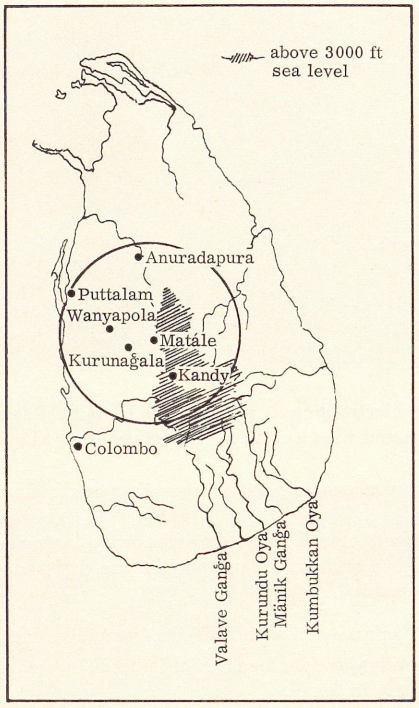

Fig. 6. Encircled area indicates regions where the incidence of thalassaemia and HbE is highest.

ination she was dyspnoeic at rest and was extremely pale. She was emaciated and had a prominent abdomen. Her liver was palpable 4 fingerbreadths below the right costal margin and her spleen 3 fingerbreadths below the level of her umbilicus.

Haemoglobin 2.1 g%, PCV 7%, MCV 77.7 μm^3, MCHC 30%, reticulocyte 0.3%, WBC 8000 mm^3 (inclusive of nucleated red cells). DC: P 36%, L 63%, E 1%. The blood picture showed marked anisopoikilocytosis, polychromasia. hypochromia, nucleated red cells and a few target cells. Tests for sickling were negative. Intraerythrocytic inclusions negative. Osmotic fragility 0.38%—0.28%. Liver function tests: serum bilirubin 0.7 mg%, thymol turbidity 6, cephalin cholesterol 4+, thymol flocculation 4+ (24 h), zinc sulphate turbidity 15 units. Alkaline phosphate 11.5 KA units. Total proteins 8.0 g%, A/G ratio 0.48. X rays of the skull were normal, that of the tubular bones and metacarpals showed thinning of the cortex, decreased density and prominent trabeculations. Haemoglobin electrophoresis showed a major fraction with mobility similar to HbE and the other similar to HbF. Alkali-resistant Hb was 3.6%.

HbE-thalassaemia was diagnosed. The child was treated with blood transfusions.

Family study: Haemoglobin analysis in the case of the father and two other siblings did not show anything abnormal. Alkali-resistant Hb was within normal limits with the absence of HbE in other members of the family including the father. The mother being dead the possiblity of the deceased mother being a carrier cannot be ruled out. Haemogram of the father: Hb 14.8 g%, PCV 45%, MCHC 33.1%, reticulocytes less than 1%, no inclusion bodies. The blood picture was normal. Osmotic fragility 0.42%—0.32%.

Discussion

In Ceylon there is a mixed population — the Veddahs, the Sinhalese, the Tamils, the Moors and the Burghers who are of European descent. The Veddahs, the original inhabitants of Ceylon (came from India 6000—3000 B.C.), now occupied the North Central, Uva and the Eastern Provinces. The Sinhalese people came from India about 500 years before the beginning of the Christian era and first settled along the river valleys of the Valave Ganga, Kiruna Oya, Manik Ganga and the Kumbukkan Oya (4). The Tamils came to Ceylon from the earliest times, about the time of the arrival of the Dravidians in South India (3rd and 4th centuries B.C.) and settled in the parts now called the Northern and Eastern Provinces. Arab shipping and merchants were established at the port of Colombo as early as 949 (5).

The haemoglobinopathies (thalassaemia and HbE) have so far been described only among the Veddahs and the Sinhalese. GRAFF *et al.* (6) first described the occurrence of HbE in the Veddahs and later LEHMANN (7) found an overall incidence of approximately 10%. HbE has also been described among the Sinhalese (82).

It is not possible to trace the racial origin of HbE in South-East Asia. It may have resulted as a mutation. As the Veddahs and the Sinhalese are partly of Bengali extraction HbE in Ceylon probably originated from Bengal where an incidence of 3.9% has been recorded, an incidence similar to heterozygous thalassaemia (9). The majority of the cases are from areas inhabited by Sinhalese of Kandyian origin, and according to SELIGMANN (4), they have a considerable amount of Veddah blood. The encircled area in Fig. 6 indicates areas where the incidence of thalassaemia and HbE is highest. The uppermost part of the encircled area includes a part of the dry zone area (Kurunegala and North and Eastern Divisions of the Matale District) which were endemic for malaria for more than 700 years. It is noteworthy that the incidence of HbE seems to be largely confined to areas which were hyperendemic to malaria before 1949 (8). It has been postulated that the Cooley gene probably originated in Central Asia. With race migration they divided into two lots one moving towards Southern Europe to countries like Greece and Italy and the other moving towards Persia and India. CHERNOFF (10) put forward an attractive hypothesis relative to the distribution of thalassaemia to mass migrations and commerce

which served to carry the genetic defect to China from a single focus in the Northern Mediterranean basin. He also quotes Brumpt to say that this gene arose from India-China and moved westwards.

α-Thalassaemia (thalassaemia-HbH disease) is the rare type of thalassaemia and is due to the inhibition of the α-chain. Thalassaemia-HbE disease is the counterpart of this kind of thalassaemia. The abnormal HbH is found only in association with thalassaemia gene and manifests itself as thalassaemia-HbH disease. The homozygous HbH nor the carrier state is found. Another distinguishing feature of this condition is the finding of intra-erythrocytic inclusions. The mode of inheritance of HbH is still not well understood. The occurrence of HbH was first described by RIGAS *et al.* (11) in a Chinese family, and is known to occur in the Thais, Filipinos and has been described in two Greek families (12) and in an English family (13).

HbH has not so far been described in Ceylon. Though the two distinguishing characteristics, the presence of a fast moving fraction (probably HbH) and intra-erythrocytic inclusions, were seen in case 1, unfortunately further crucial tests could not be carried out. Some members of the family had an increase in the alkali resistant Hb but no abnormal Hb was detected. Inclusion bodies without HbH have been reported in Thailand by MINNICH *et al.* (14) in three cases presenting as thalassaemia-HbH disease. Thalassaemia-HbH disease varies in severity though it is less severe than thalassaemia major. Thalassaemia-HbH patients are prone to haemolytic crisis and this is probably what happened to case 1, ending fatally.

In *β-thalassaemia* there is besides the increase in the alkali-resistant Hb, an increase in the A_2 fraction unlike in α-thalassaemia. The clinical picture of the homozygous form described here, differs in no way from that described by DE SILVA *et al.* (8) and others. Symptoms appear within the first year of life, are severe with gross enlargement of the liver and spleen. Majority of them died before the age of two years. The red cells showed increased resistance. The blood picture reveals a large number of nucleated red cells in various stages of maturation and a varying number of target cells. Radiological changes may be minimal and if found usually occur in the long bones and in the skull. Case 2 showed an increase in HbF. Increase in HbF is a well established abnormality in thalassaemia and is regularly found in homozygous forms.

Values are usually high (approximately between 90%—20%). Cases 2 and 3 are obviously homozygous forms. The mother of case 3 had an increase in A_2 while the father showed no abnormality.

The heterozygotes show considerable indivdual variation in the expression of the trait. The majority of the cases show little besides mild anaemia with hypochromia and a few or no target cells. In the heterozygous state however HbF may not be significantly raised. Following the identification of A_2 by KUNKEL and WALLENIUS (15) this fraction has become important in the diagnosis of thalassaemia, more so in the heterozygous states (thalassaemia minor and carrier states) where it constantly raised (*vide* family of case 2).

Thalassaemia with β-chain anamolous HbE results from interaction of the thalassaemia gene with the gene for HbE. Like thalassaemia-HbH disease the condition presents a wide range of severity as illustrated by cases 4 and 5. Haematological and radiological changes may be indistinguishable from that of thalassaemia major.

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Summary

A case of thalassaemia-HbH disease is described for the first time from Ceylon. The thalassaemia syndromes in Ceylon now include thalassaemia (major, minor and carrier state), thalassaemia-HbH disease and thalassaemia-HbE disease, which are discussed.

Zusammenfassung

Es wird erstmals über einen Fall von Thalassämie-Hb H-Krankheit aus Ceylon berichtet. Die Thalassämie-Syndrome in Ceylon umfassen nun Thalassämie (major, minor und Merkmalsträger), Thalassämie-Hb H-Krankheit und Thalassämie Hb E-Krankheit, die diskutiert werden.

Résumé

Un cas de thalassémie HbH est décrit pour la première fois à Ceylan. Les syndromes thalassémiques comprennent actuellement à Ceylan la thalassémie (majeure, mineure et les états de porteurs de gènes), ainsi que les maladies thalassémiques avec HbH et HbE qui sont discutées ici.

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