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Hereditary Elliptocytosis Associated with Beta-Thalassaemia and a Variant of Rh (D)

A Study in a Sinhalese Family

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Abstract. A Sinhalese family with hereditary elliptocytosis is been described. Three of the members had thalassaemia trait and 2 had CD^{u}e chromosome. There was no abnormality due to the elliptocytosis nor to the presence of the thalassaemia trait. Both genes had not interacted. The osmotic fragility was markedly decreased in 2 members before and after incubation. Two members did not show any haptoglobin bands.

Key Words
D^{u} blood group
Elliptocytosis
Thalassaemia

Hereditary elliptocytosis is an uncommon disorder and its incidence in Ceylon is not reported. Thalassaemia, however, is not uncommon [1–3]. Both β- and α-thalassaemia are known to exist, while instances of interaction of thalassaemia with Hb E are also described [3, 4]. Multiple inherited erythrocytic abnormalities have been known to occur in families. COHEN *et al.* [5], CUNNINGHAM and VELLA [6] and SWARUP *et al.* [7] described the occurrence of hereditary spherocytosis with thalassaemia. Beta-thalassaemia with hereditary elliptocytosis has also been reported [8–10]. Hereditary elliptocytosis with Hb C [11], with Hb S [12] and with hereditary persistence of foetal haemoglobin [13] have been described.

This is a study of a Sinhalese family whose members showed a combination of hereditary elliptocytosis, β-thalassaemia trait and a variant D^{u} antigen in the Rh system.

Methods

The methods used in the haematological investigations have been described elsewhere [4]. Electrophoresis of haemoglobin was carried out using paper at pH 8.6 (veronal) and 8.9 (tris buffer). Estimation of Hb A_2 was carried out on cellulose acetate strips [14]. Foetal haemoglobin was estimated by alkali-denaturation technique [15]. For demonstration of erythrocyte inclusion bodies brilliant cresyl blue vital staining was used. Haptoglobin types were determined by starch gel electrophoresis [16]. Screening test for G6PD deficiency was done by using brilliant cresyl blue dye decolorization test [17]. Total glutathione levels of the red cells were estimated by the modified nitroprusside method [18]. The variation in the agglutination reaction were further scored by titrating with 13 different incomplete anti-Rh (D) sera free from anti-C with the papain treated red cells of all the members of the family. One of incomplete anti-D was obtained from a Cde/cde (R′r) patient. Three Rh sera were from Ortho Diagnostics, USA, and the rest were locally prepared. All Rh sera had Rh(D) specificity except one which was anti-DE.

Case Reports

Case S3, propositus. A Sinhalese male, aged 22 years, was admitted for investigation on 14. 11. 1969 with a history of fever of 4 days duration and an enlarged spleen. He had just returned from an area where malaria was endemic. He had been in good health prior to this.

Examination revealed a febrile individual. He had no other abnormality except 2 fingerbreadths palpable spleen below costal margin. The same evening he had a rise in the temperature with chills and rigor and was clinically thought to have malaria and was given anti-malarial drugs, following which he remained afebrile.

Haemoglobin 10.2 g%, PCV 27%, MCV 92.5, MCHC 30.3%, reticulocytes 0.4%, WBC 9,400 μm^3, P 70%, L 26%, E 4%. Large number of elliptocytes (about 75%), mild aniso-poikilocytosis and a fair number of target cells (fig. 1b). Paper electrophoresis revealed no abnormal haemoglobin, Hb A_2 was increased (3.8%). There was no increase in the alkali-resistant haemoglobin (1.33%). G6PD activity was normal and total glutathione level in the red cells was 30.0 mg%. No haptoglobin bands were detected in the serum on starch gel electrophoresis on repeated examination. Agglutination reaction with 2 routine anti-Rh(D) sera gave slightly weaker reaction than in the mother (S2) and the sister (S4).

Diagnosis: Hereditary elliptocytosis and thalassaemia trait.

Figure 2 shows the family tree.

Case S1, father, aged 50 years, was asymptomatic. Haematological, biochemical and liver function tests are shown in tables I and II. Large number of elliptocytes and target cells were seen (fig. 1a). Haemoglobin A_2 was increased (4.22%). The red cells gave strikingly weak positive to positive (+) agglutinations with 13 different incomplete anti-D sera used routinely in the laboratory as compared to other members of the family (S2, S3, S4) and control samples. Further, the titration

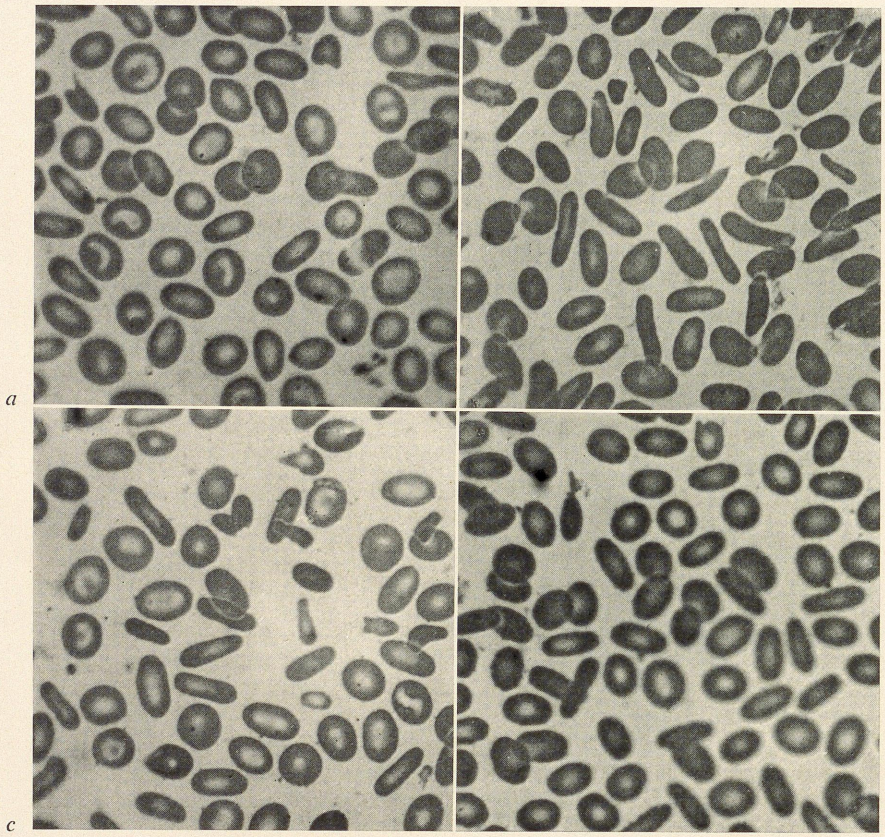

Fig. 1. Peripheral blood of father (S1), propositus (S3), sisters (S4, S5).

score with selected anti-Rh(D) sera showed on the average poor score 7 with S1 cells, whereas S2, S3, S4, R_1r and R_1R_1 controls gave 25, 20, 23, 30 and 35 score values respectively. Control fresh cells showed higher scores. The red cells of S1 (father) exhibited a variant of D and is designated as D^u, since the reactions and the scores were consistently weak as compared to other members of the family (S2, S3 and S4). The case, therefore, appears to be similar to one described by DUNSFORD [19], where his D subject consistently gave weak agglutination with most Rh sera used. A same variant was found in his family.

In few instances Cde(R′) chromosome has been reported to suppress the expression of D, thereby apparently behaving as a high grade D^u and may give weak reactions. Such a possibility could arise if the S1 has an extremely rare genotype Cde/cDe(R′Ro). But the ccd dee phenotype of S5 (sib) and CcD ee of S2 (mother) ruled out the possibility of S1 (father) being a rare type Cde/cDe(R′Ro).

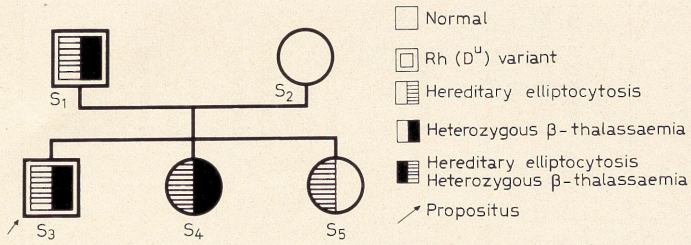

Fig. 2. Family pedigree showing hereditary elliptocytosis, β-thalassaemia trait and Rh(D^u) variant.

Diagnosis: Hereditary elliptocytosis, thalassaemia trait and Rh(D^u) variant.

Case S2, mother, aged 40 years, had no abnormality. The bloodpicture showed mild hypochromia. Results of the other investigations are shown in tables I and II.

Diagnosis: Normal.

Case S4, sister, aged 24 years, had no symptoms but her mucous membranes were pale. Her spleen was not palpable. Besides moderate anemia (table I), the blood picture revealed a large number of elliptocytes, moderate anisopoikilocytosis, target cells, hypochromia and microspherocytes (fig. 1c). The haematological and biochemical data are summarised in tables I and II. Haemoglobin A_2 was increased (4.02%). No haptoglobin bands could be detected on starch gel electrophoresis.

Diagnosis: Hereditary elliptocytosis, thalassaemia trait and normal Rh subtype CDe/cde(R_1r).

Case S5, sister, aged 20 years, had no complaints and she had no clinical or haematological abnormality except her red cells showing elliptocytosis (fig. 1d). Haemoglobin A_2 was normal (2,06%).

Diagnosis: Hereditary elliptocytosis.

The propositus (S3) and the father (S1) had decreased fragility both of fresh and incubated blood. The sister (S4) showed some of the cells resistant both before and after incubation. The other sister (S5) and mother (S2) had normal fragility. X-ray of the skull and hands were normal in all of them.

Discussion

The blood picture and the results of the haemoglobin analysis of the propositus, the father and the elder of the 2 sisters revealed that they had elliptocytosis with thalassaemia trait. Serologically S1 was found to be D^u (CD^{u}e/cde) and family investigations revealed S3 having a rare genotype CD^{u}e/CDe ($R_1{}^uR_1$). S1, S3 and S4 had normal alkali-resistant haemoglobin but elevated haemoglobin A_2 levels. In Ceylon, there is a preponderance of A_2-thalassaemia in families of patients with β-thalassaemia major [20]. In the diagnosis of thalassaemia trait, deter-

Table I. Haematological data

Case	Age years	Hb g%	PCV %	MCV μm^3	MCHC %	Reticulocytes %	Red cell morphology						Osmotic fragility, fresh and incubated blood
							anisocytes	poikilocytes	elliptocytes	spherocytes	hypochromia	target cells	
S1													
Father	50	11.1	39	97.5	28.5	1.2	+	+	++	0	+	++	markedly decreased
S2													
Mother	40	12.4	42	95.0	29.3	0.4	0	0	0	0	+	0	normal
S3													
Propositus	22	10.2	37	92.5	30.3	0.4	+	+	+++	0	0	++	markedly decreased
S4													
Sister	21	9.4	35	97.2	26.9	0.4	++	+	+++	+	+	++	partly decreased
S5													
Sister	20	14.4	46	93.9	31.3	0.4	0	0	++	0	0	0	normal

Table II. Results of liver function and other investigations

Case	Liver function tests						Hb-A_2 fraction %[1]	Alkaline resistant Hb, %	Glutathione mg %[2]	Haptoglobin type	Blood groups	
	bilirubin mg %	ZnSo4 turbidity	thymol turbidity	thymol flocculation	alkaline phosphatase KA units	cephalin cholesterol					ABO phenotype	Rh genotype
S1	0.4	4	–	nil	8	+2	4.22	0.875	28.5	2–1	O CcD^{u}ee,	CD^{u}e/cde, ($R_1{}^{u}r$)
S2	–	–	–	–	–	–	1.96	1.96	32.0	2–1	B CcDee	CDe/cde, (R_1r)
S3												
Propositus	0.3	12	2	nil	9	+1	3.80	1.33	30.0	0–0	O CCD^{u}ee,	CD^{u}e/CDe, ($R_1{}^{u}R_1$)
S4	0.6	24	3	nil	10	+ + ½	4.02	1.76	34.0	0–0	O CcDee,	CDe/cde, (R_1r)
S5	0.4	19	2	nil	13	+2	2.06	0.84	29.0	2–2	B ccddee,	cde/cde, (rr)

[1] Normal range 1.9–3.1 %. Sickling absent. G6PD (screening test). Normal activity.
[2] Mean value 40.0 mg %. Direct Coombs tests negative. No inclusion bodies.

mination of Hb A_2 is more reliable. According to WASI *et al.* [21], in the presence of iron deficiency anaemia, the relative amounts of Hb A_2 may be lowered considerably in normal people and thalassaemia trait and may be so lowered as to miss the diagnosis of thalassaemia trait. Our cases had low MCHC and this may have been complicated by iron deficiency.

S1, S3 and S4 did not have any clinical abnormality and there was no evidence of haemolysis as judged by the normal serum bilirubin and reticulocyte count, though S4 was somewhat anaemic. The youngest sister (S5) was a case of inherited elliptocytosis. In the great majority of cases reported elliptocytosis had been a harmless anomaly.

The fact that 75% of the cells showed elliptocytosis cannot be explained only by the presence of β-thalassaemia trait in S1. Further, the presence of elliptocytosis alone in S5 in the absence of other haematological abnormality supported the finding of elliptocytosis in the father (S1) to be truly hereditary elliptocytosis and not the one which commonly is associated with thalassaemia trait. In S4 there were microspherocytes and this has been known to occur with hereditary elliptocytosis [22]. The blood films of S1, S3 and S4 showed an increased number of target cells, suggestive of thalassaemia trait in addition to the elliptocytosis. The former was confirmed by haemoglobin analysis.

In this family the gene for elliptocytosis did not travel with R_1 (CDe), R_1^u (CD^ue), or r (cde) since all 3 siblings did not have either R_1^u or r but showed elliptocytosis inherited from R_1^ur (CD^ue/cde), father. This family, therefore, deviated from the usual linkage reported by several workers and analysed by MORTON [23].

Hp (2–1) X Hp (2–1) mating (S1 X S2) has produced no detectable haptoglobin bands in S3 and S4 on starch gel electrophoresis. 2 genetic conditions namely thalassaemia trait and elliptocytosis in the propositus (S3) and his father (S1) may not be responsible for the absence of Hp bands, since S1 showed a distinct (2–1) type on electrophoresis but not S3 both having elliptocytosis and thalassaemia. Again the sister (S4), a thalassaemia carrier, did not show any Hp bands. It may seem that the absence of Hp bands in S3 and S4 is independent of other genetical conditions observed in this family. It may have been brought about by further lowering of the Hp levels in their blood by some haemolytic processes. It is found in some populations of SE Asia and New Guinea that low haemolytic episodes may not enable the Hp phenotype to be assigned by usual typing using starch gel electrophoresis [24].

S1 and S3 in this study had markedly reduced fragility both of fresh and incubated cells. This marked resistance could be explained by the coexistence of thalassaemia. Thalassaemia itself causes increased resistance. S4 however, had only a part of the cells showing decreased fragility, a greater part fell within the normal range. She had microspherocytes and this could account for the greater part of the curve lying within the normal range. Though the fragility is normal in uncomplicated elliptocytosis, increased resistance may be suggestive of the presence of another associated abnormality like thalassaemia or a haemoglobinopathy. AVERY [25] described a case of Hb C and elliptocytosis whose osmotic fragility was normal or near normal before incubation and shifted in the direction of increased resistance on incubation.

Cases have been described of elliptocytosis associated with oxycephaly [26] and defects of the lateral incisors [27]. None of our cases had these defects. PENFOLD and LIPSCOMB [28] described a Jewish family with hereditary elliptocytosis and hereditary haemorrhagic telangiectasia.

Hereditary elliptocytosis is inherited as a Mendelian dominant and is equally common in males and females. According to MORTON [23] there may be more than one gene responsible for the morphological appearances of the red cell. Interesting problems arise when two or more genes for inherited haemolytic disorders co-exist in the same population and interact, so that a person inheriting both mutant gene is at a disadvantage. In SE Asia for example, β-thalassaemia and Hb E are both present and the presence of the 2 genes in an individual leads usually to a moderately severe blood disorder. In our cases the elliptocytic trait has been associated with the thalassaemia trait but the individuals were seemingly unaffected. It is apparent that in these cases no interaction has occurred. Similarly in the family with hereditary persistence of foetal haemoglobin and elliptocytosis, no evidence of interaction was seen in individuals heterozygous for these genes [13]. Elliptocytosis associated with Hb C [25] and Hb S [12] were found in a case with interaction between an inherited spherocytic gene and thalassaemia gene to the individuals disadvantage.

According to the reports of association of hereditary elliptocytosis with β-thalassaemia there seems to be no evidence to show mutual enhancement of the involved genes [8, 9, 29]. But in the family reported by PERILLIE and CHERNOFF [10] there is evidence of haemolytic anaemia being the result of the summation of the clinical effects of the genes for hereditary elliptocytosis and β-thalassaemia in the same individual. In

our cases there seems to be no evidence of any overt haemolytic anaemia in the individuals carrying the genes for elliptocytosis and β-thalassaemia. The fact that 3 of the 4 members in this family showing increased number of elliptocytes had also β-thalassaemia does not necessarily indicate that this abnormal morphology of the red cells is associated with thalassaemia. This is borne out by the fact that the other member in the family showing elliptocytosis is found to be free of β-thalassaemia trait with normal amount of Hb A_2. From the limited genetic data available there is reasonably good evidence that the genes for β-thalassaemia and hereditary elliptocytosis in one family reported here are not allelic.

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