Two families in Sri Lanka with southeast Asian ovalocytosis

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Introduction

Inherited defects of the red cell membrane which lead to abnormal red cell morphology, indices and osmotic fragility are not uncommon. Some defects cause clinically significant haemolysis whereas others run a relatively benign course. This paper emphasises the importance of

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being aware of one such benign disorder. Southeast Asian ovalocytosis (SAO), also known as stomatocytic elliptocytosis of Melanesians, has a unique red cell morphology in the blood film [1]. It is most commonly seen among Melanesians in Papua New Guinea, in some population groups in Indonesia and Malaysia, and in Malaysian aboriginals [2,3,4]. We report two families with SAO in Sri Lanka.

Case 1

A 7-year old boy was admitted to the Lady Ridgeway Hospital with a viral fever. A blood picture done as a preliminary investigation showed over 30% of the red cells to be large ovalocytes with some showing a central slit or transverse ridge. These features are characteristic of SAO. Screening of family members revealed similar features in the blood picture of his sister (only sibling), father and paternal grandmother whose father was an immigrant from Malaysia (Figure 1, Table 1). The blood picture of the boy's mother was normal. There was no evidence of haemolysis in any of the affected members.

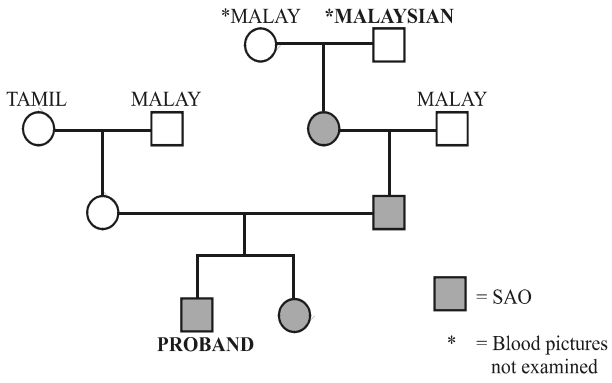

Figure 1. **Family tree of the first case.**

Table 1. **Comparison of red cell indices of family members of first case**
An increase in the variation of red cell size is indicated by a high red cell distribution width or RDW (normal < 13.2%), which is probably due to the ovalocytes.

	Age (years)	*RBC ($\times 10^{12}$/L)*	*Hb (g/dL)*	*RDW (%)*
Proband	7	4.65	12.9	14.2 (high)
Sister	3	4.41	12.6	13.8 (high)
Father	37	4.71	14.9	15.3 (high)
Mother	36	4.78	13.7	12.0
Grandmother	65	3.7	12.1	14.6 (high)

Hb = haemoglobin, RBC = red blood (cell) count, RDW = red cell distribution width

Case 2

A 30-month old boy had been investigated for a haemolytic anaemia since birth at Base Hospital, Horana. He was pale, icteric and had hepatosplenomegaly with a haemoglobin of 6.5 g/dL, a reticulocyte count of 10.5% and a serum bilirubin of 170 µmol/L. The blood picture showed red cells which were markedly hypochromic and microcytic, a population of large ovalocytes, some with central ridges, polychromasia and normoblastaemia. The white cells and platelets were normal. Haemoglobin electrophoresis and alkaline denaturation test (HbF - 21.3%) confirmed that he had beta thalassaemia major together with features characteristic of SAO. As he was the only live child, his parents were screened for both conditions. His father, a Sinhalese from Anuradhapura was found to have a beta thalassaemia trait (HbA_2 - 5.8%) with SAO. His mother a Sinhalese from Kurunegala had a beta thalassaemia trait (HbA_2 - 5.1%) with no evidence of SAO (Figure 2, Table 2).

The child is being managed with on demand blood transfusions and parenteral iron chelation therapy.

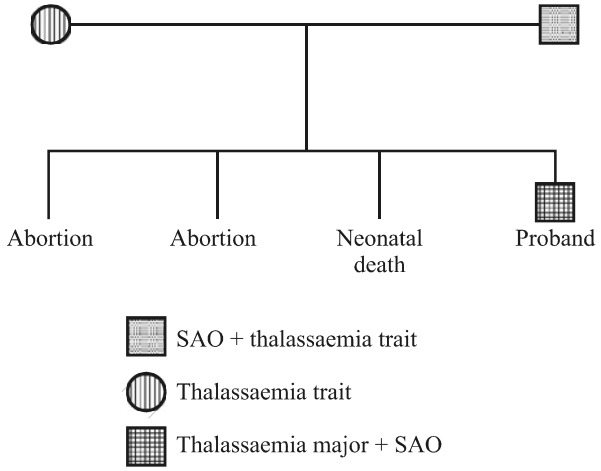

Figure 2. **Family tree of the second case.**

Discussion

Southeast Asian ovalocytosis has an autosomal dominant inheritance. It results in red cells that are rigid and hyperstable (rather than unstable) giving rise to the unique red cell morphology [2]. The red cell membrane is composed of a phospholipid and cholesterol bilayer and a number of membrane protiens. These proteins have been categorised as integral membrane proteins and peripheral membrane proteins, and assigned specific names. These are based on the mobility of each protein on a sodium dodecyl sulphate polyacrylamide gel electrophoresis.

The protein that is defective in SAO (anion exchanger 1) is identified on band 3 when stained with Coomassie brilliant blue stain [5]. Two mutations that cause abnormalities in the band 3 protein have been identified

Table 2. **Comparison of red cell indices (*Proband was after blood transfusion)**
The low indices of the father and mother of this child are consistent with thalassaemia minor. The significant elevation of the father's RDW is compatible with the presence of large ovalocytes.

	Age (years)	*Hb (g/dL)*	*RBC ($\times 10^{12}$/L)*	*MCV (fL)*	*MCH (pg)*	*MCHC (g/dL)*	*RDW (%)*
Proband*	2.5	9.5	3.2	81.6	29.8	35.8	12.0
Father	38	9.8	4.27	77.0	22.9 (L)	29.8	16.6 (H)
Mother	32	8.5	4.05	69 (L)	21.0 (L)	30.4	14.5 (H)

H = High, L = Low, Hb = haemoglobin, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, MCV = mean corpuscular volume, RBC = red blood (cell) count, RDW = red cell distribution width

in patients with SAO. Homozygosity for these mutations are thought to lead to embryonic lethality while the heterozygous state results in the formation of abnormal erythrocytes which exhibit increased binding of band 3 protein to Ankyrin (band 2.1 protein), increased tyrosine phophorylation of band 3 protein, inability to transport sulfate anions and a markedly restricted lateral and rotational mobility [2,3]. Other abnormalities associated with SAO are reduced osmotic fragility of red cells, resistance to shape change by echinocytic agents and a reduced expression of many red cell antigens.

Another remarkable feature of SAO erythrocytes is their resistance to invasion by several strains of malarial parasites, including *Plasmodium falciparum* and *P. knowlesi*, particularly against heavy infections and cerebral malaria [2,3]. Band 3 serves as one of the receptors for the malarial parasite, as evidenced by inhibition of invasion in vitro [2]. The abnormal band 3 protein may not function as a receptor for the malarial parasite.

The diagnosis of SAO can be made accurately on a blood film stained with Romonowsky stain [6,7]. The finding of 30% or more of oval shaped red cells with stomatocytes, with a notable absence of clinical and laboratory evidence of anaemia and haemolysis is highly suggestive [2]. There is also a minor population of macro-ovalocytes, many of which are stomatocytic. The stoma is two in each cell. It may be longitudinal, transverse, V-shaped or Y-shaped [1]. The haemoglobin, mean corpuscular value (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and the reticulocyte count are normal. A useful screening test is the demonstration of the resistance of ovalocytes or their ghosts to changes in shape produced by treatment that produces spiculation in normal cells, such as overnight incubation of red cells [2]. Rapid genetic diagnosis can be made by demonstrating a shorter band (27 base pair deletion) compared to a normal control [2,7].

Conclusion

Diagnosis of SAO can be made on a blood picture or by DNA analysis. Awareness of the presence of this red cell disorder in Sri Lanka, with a multi-racial population and possible preservation of the gene in areas endemic for malaria, is important.

References

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