Hereditary ovalocytosis in Malays

E. George FRCPA*, N. Mohandas DSc**, G. Duraisamy FRCPA**, N. Adeeb MRCOG*, Z.A. Zainuddin MP*, M.S. Teng*** and R. Vimala***

**National University of Malaysia, Kuala Lumpur*
***National Cancer Institute, University of California, USA*
****National Blood Services Centre, Kuala Lumpur*

Summary
Hereditary ovalocytosis is present in 5.1% of Malays. Malays with hereditary ovalocytosis are asymptomatic and show no overt haemolysis or splenomegaly. Ektacytometric studies of the peripheral blood showed the red cells are markedly rigid. The phenotypic expression is that of stomatocytic ovalocytosis with an autosomal dominant pattern of inheritance.

Key words: Hereditary ovalocytosis; Malays; Stomatocytosis.

Introduction
Since the first description of hereditary ovalocytosis in Malayan aborigines[1] 1965, high frequencies of hereditary ovalocytosis have been reported in South East Asia.[2,3] Biophysical studies showed a strong correlation between increased membrane rigidity and decreased malarial parasite invasion in hereditary ovalocytosis in the Malayan aborigines.[4] The literature currently available describes hereditary ovalocytosis in the Malayan aborigines and minimal data are available on its findings in the Malays. The following is a report of the frequency of hereditary ovalocytosis in the Malays with haematological data, Rh blood types and biophysical data.

Materials and Methods
From May to October 1987, 665 consecutive cord blood samples from Malay children born at the General Hospital, Kuala Lumpur were screened for hereditary ovalocytosis. 1 millilitre of cord blood was collected in E.D.T.A. and blood smears were stained by routine haematological procedures.[5] A careful study of the red cells were done by two independent investigators. The presence of ovalocytes in 40% of the red cells examined was considered as minimum for diagnostic purposes.[6] Venous blood from 101 adult subjects with hereditary ovalocytosis were obtained for complete blood counting and Rh genotyping by standard methods.[5] 2093 Malay blood donors formed the control group of the Rh genotypes. 10 millilitres of blood from 7 cases of hereditary ovalocytosis were drawn into A.C.D. and airfreighted at 4-6^{o}C on ice to San Francisco. The delay from Kuala Lumpur from the time blood was drawn to the time of analysis was 36 hours. The methods used for the determination of the deformability index of the red cell membrane and ghosts and for the two dimensional analysis of spectrin have been described elsewhere.[7,8] 6 millilitres of venous blood freshly drawn from 12 cases of hereditary ovalocytosis were subjected to the osmotic fragility test, at room temperature (23-28^{o}C) and lysis was read using a spectrophotometer at a wavelength of 540nM.[5]

Statistical Considerations: Students T test was used to compare the significance of the laboratory data (Abstat^{Tm} dBase II Statistical Package).

Table I

Red cell parameters and serum bilirubin levels in Malays with hereditary ovalocytosis (n = 101)

	Female	Male
	(50)	(51)
Hb gm/dl	13.2 (11.2 - 15.2)	15.9 (12.6 - 19.2)
RBC ($X10^{12}$/L)	4.4 (3.2 - 5.5)	5.2 (3.8 - 6.7)
HCT (%)	39 (38 - 44)	46 (40 - 56)
MCV (fl)	89.2 (73.6 - 104.8)	89.1 (73.6 - 104.6)
MCH (pg)	30.0 (23.8 - 36.2)	30.7 (23.5 - 37.9)
MCHC (%)	33.6 (30.2 - 37.0)	34.3 (29.1 - 39.5)
S. Bilirubin (mmol/L)	9 - 20 mmol/L	

Results

Haematological data are presented in Table I. No significant differences were seen in the red cell parameters in subjects with hereditary ovalocytosis and the normal control group ($p < 0.100$). Hereditary ovalocytosis in the Malays show presence of ovaloyctes in the peripheral blood which are egg shaped. The red cell membranes fold in blood smears producing a transverse "stoma" leading to their designation as stomatocytic hereditary ovalocytosis. (Figures 1,2). The ovalocytes are osmotically resistant with the median corpuscular fragility (MCF, the concentration of saline causing 50% lysis) at 3.9gm/dl (normal range 4.0 – 4.5 g/L). Cord blood studies showed the presence of stomatocytic ovalocytes in 34 (5.1%) of the blood smears examined.

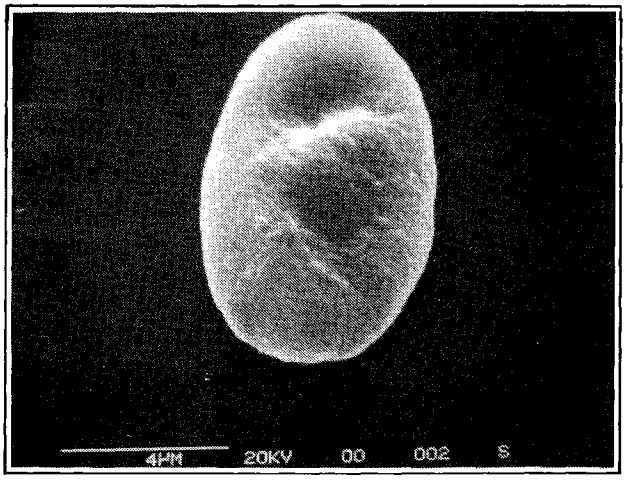

Fig 1 Electron scan of a cell with stomatocytic ovalocytosis.

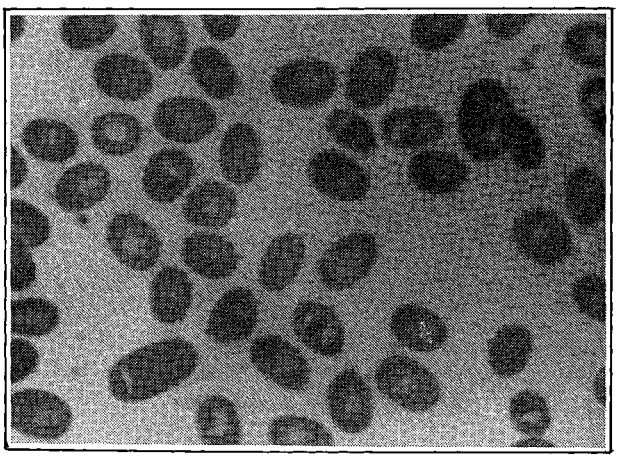

Fig. 2 Electron scan of a cell with stomatocytic ovalocytosis

Table II

Probable Rh genotypes in Malays with hereditary ovalocytosis

Phenotype	Probable Rh Genotype	Ovalocytosis n = 101		Control n = 2093	
		No.	%	No.	%
CCDe̅e̅	CDe̅/CDe̅	62	61.4	1243	59.4
Cc̅De̅e̅	CDe̅/c̅de̅	14	14.0	278	13.3
Cc̅DEe̅	CDe̅/cDE	16	16.0	435	20.8
c̅c̅DEE	cDE/cDE	1	0.9	34.0	1.6
CCDEe̅	CDe̅/CDE	5	5.0	46	2.2
c̅c̅DEe̅	cDE/cde	1	0.9	42	2.0
c̅c̅De̅e̅	c̅De̅/cDe̅	0	0	11	0.5
Cc̅DEE	c̅De̅/CDE	0	0	2	0.1
CCDEE	CDE/CDE	1	0.9	2	0.1
Cc̅D^{u}e/de̅e̅	CD^{u}e/c̅de̅	1	0.9	0	0

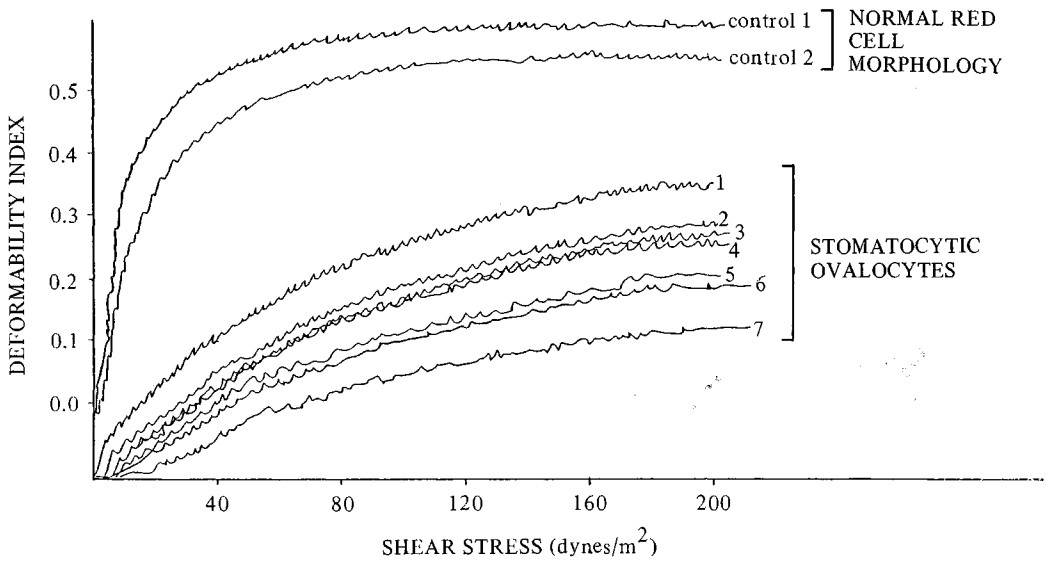

Fig. 3: Deformability index versus shear stress for stomatocytic ovalocytosis for seven Malays.

The pattern of inheritance is autosomal dominant in 9 cases where complete kindred studies were possible. The probable Rh genotypes seen does not differ from the gene frequencies in the Malay population suggesting that the ovalocytic abnormality does not segregate with the Rh genotype ($r = 0.249$, p 0.100) (Table 1). The Rh D variant (D^u) expression was seen in 1 (0.9%). Ektacytometric studies of the red cell membranes and ghosts showed the cells are markedly rigid (Figure 3). Two dimensional tryptic studies of spectrin did not show any abnormalities.

Discussion

Hereditary ovalocytosis represents a heterogenous group of disorders which differ in clinical expression, red cell morphology and molecular pathology. Our study shows the abnormality is present in 5.1% of the Malays in this country. Hereditary ovalocytosis does not cause overt haemolysis or splenomegaly. The condition is usually picked up as an incidental finding from routine blood film examination.

Ovalocyte formation may result from an integral membrane protein defect or an abnormality of protein interaction. The molecular basis of hereditary elliptocytosis due to membrane protein 4.1 deficiency[9] and an abnormal spectrin have been described.[8] Ektacytometric studies of the peripheral blood showed the red cells are markedly rigid indicating a skeletal protein defect which has yet to identified.

Hereditary ovalocytosis showing autosomal dominant inheritance with the abnormal gene linked to the Rh blood group genes on the same chromosomes has been described. In these families the elliptocytic deformity of the cells segregates with the Rh blood group genotype.[13] In the Rhesus blood group, Cde has been reported to suppress the expression of D behaving as a high grade D^u [11,12] The D^u expression however was seen in only one case. In the Malays the ovalocytic abnormality does not segregate with the Rh genotype. The phenotypic expression is that of stomatocytic ovalocytosis which also has been described in the Melanesians. The pattern of inheritance is autosomal dominant in contrast to the findings in the Melanesians.[10]

Further studies are necessary to describe the underlying molecular defect of hereditary stomatocytic ovalocytosis in the Malays.

References

1. Lie-Injo LE. Hereditary Ovalocytosis and Haemoglobin E Ovalocytosis in Malaysian Aborigines. Nature 1965; 208 : 1329.

2. Lie-Injo LE, Fix A, Bolton JM, Gilman RH. Haemoglobin E-Hereditary Elliptocytosis in Malayan Aborigines. Acta Haematol 1972; 47 : 210.

3. Amanto D, Booth PB. Hereditary Ovalocytosis in Melanesians. Papua New Guinea Med J 1977; 20 : 26–28.

4. Mohandas N, Lie-Injo LE, Friedman M and Mak JW. Rigid Membranes of Malayan ovalocytes: A Likely Genetic Barrier Against Malaria. Blood 1984; 6 : 1385–1392.

5. Dacie J and Lewis SM. Practical Haematology 1975; 5th Ed., Edinburgh, Churchill and Livingstone.

6. Florman AL, Wintrobe MM. Human Elliptical Red Corpuscles. John Hopkins Hosp. Bull. 1938; 63 : 209 – 211.

7. Mohandas N, Clark M, Health B, Rossi M, Wolfe L, Lux SE and Shohet SB. A Technique to Detect Mechanical Stability of Red Cell Membranes: Relevance to Elliptocytic Disorders. Blood 1982; 59 : 768–774.

8. Marchesi SL, Knowles WJ, Marrow JS, Bologna MB and Marchesi VT. Abnormal Spectrin in Hereditary Elliptocytosis. Blood 1986; 1: 141–151.

9. John C, Mohandas N, Tchernia G, Kan YW. Molecular Basis of Hereditary Elliptocytosis Due to Protein 4.1 Deficiency. N. Engl. J Med 1986; 11 : 680–684.

10. Marchesi SL, Knowles WJ, Marrow JS, Bologna M and Marchesi VT. Abnormal Spectrin in Hereditary Elliptocytosis. Blood 1986; 67 : 141–151.

11. Nagaratnam N, Siripala KA, Attapu AM, Undevia JV and Sukumaran PK. Hereditary Elliptocytosis Associated with Beta Thalassaemia and A Variant of Rh(D). Acta Haemat 1971; 46 : 232–241.

12. Morton NE. The Detection and Estimation of Linkage Between the Genes for Elliptocytosis and the Rh Blood Type.Amer. J. Hum. Genet. 1956; 8 : 80–96.

13. Hoffbrand AV and Lewis SM. Postgraduate Haematology. 1981; 2nd Ed., London, William Heinemann, Medical Books Ltd.