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THE OSMOTIC FRAGILITY OF RED CELLS IN HEREDITARY STOMATOCYTIC ELLIPTOCYTOSIS.

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Summary

Hereditary stomatocytic elliptocytosis is not an uncommon inherited disorder found among Malaysians of Malay descent. We have studied the osmotic fragility of elliptocytes by the standard osmotic fragility test and the acidified glycerol lysis-time test. We have found that elliptocytes are more fragile than normocytes. The acidified glycerol lysis-time of elliptocytes is shortened in comparison to normocytes. The difference is probably due to the underlying membrane defect.

Keywords: Elliptocyte, Red cell osmotic fragility.

INTRODUCTION

Hereditary elliptocytosis (HE) is an inherited disorder. The appearance is distinctive and may occur in three forms: (a) the common HE as shown in Fig. 1 (obtained from an Indian patient), (b) the spherocytic HE and (c) the stomatocytic HE as shown in Fig. 2 (obtained from a Malay patient.[1]) We report the results of a study of osmotic fragility on the stomatocytic HE.

MATERIALS AND METHODS

Samples

Thirty consecutive samples of blood collected in EDTA sent for full blood picture in the haematology laboratories of the University with >70% elliptocytes were studied within four hours of collection. 30 consecutive samples of blood collected similarly with >70% normochromic, normocytic red cells were used as control. 8 consecutive samples of blood from patients who were known to have congenital spherocytosis with >70% spherocytes in the peripheral blood were also studied.

Methods

The method for testing osmotic fragility was according to Parpart *et al.* (1947).[2] Hypotonic saline buffered to pH 7.4 was used and the blood was added to the hypotonic solutions in the proportion of 1 to 100. The test was carried out at room temperature ($26 - 30^{o}c$) and lysis was read using a spectrophotometer at a wavelength setting of 540 nm.

The same samples were subjected to the glycerol lysis-time test according to Zanella *er al.* (1980).[3] This method detects osmotically fragile cells and uses one tube rather than multiple tubes as in the former test by Parpart. Phosphate buffered saline at pH 6.85 was used. The rate of haemolysis was measured by the rate of fall of turbidity of the reaction mixture using a spectrophotometer at a wavelength setting of 625 nm.

RESULTS

The patient characteristics according to red cell type are shown in Table 1. Table 2 and Fig. 3 express the findings of the osmotic fragility test. The results of the Glycerol lysis-time test are summarized in Table 3 and Fig. 4.

DISCUSSION

Stomatocytic HE is not an uncommon genetic disorder found among Malaysians of Malay descent. Patients normally show no evidence of haemolysis. They are usually picked up as an incidental finding from a routine blood film examination. This disorder is common in the Melanesian population but rare in other parts of the world. It was first reported by Amato in 1975.[4]

Our study shows that the fragility curve of elliptocytes is closer to that of normocytes but the mean GLT50 is intermediate between that of normocytes and spherocytes. There is, however, overlap of mean GLTSO between normocytes and elliptocytes and also between elliptocytes and spherocytes. Both tests have shown that elliptocytes are more fragile than normocytes. This property of elliptocytes is better shown by the acidified glycerol lysis-time test. Thus the acidified glycerol lysis-time is a useful screening test for elliptocytes rather than the standard osmotic fragility test. How-

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ever it is not specific as spherocytes also have a shortened GLT50.

The abnormal shape and increased fragility of the red cells in HE is due to a molecular defect in the membrane proteins which constitute the submembrane skeleton or which hinge the skeleton to the membrane. Various protein abnormalities have been reported. These include abnormal spectrin, abnormal protein 4.1 and absence of one or more minor sialoglycoproteins. Relationships among these protein deficiencies and the development of the clinical syndrome of HE are not clearly established.[4,5]

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TABLE 1

PATIENT CHARACTERISTICS

Grouping of Red Cells	Mean Age (yr) and (Range)	Number of Patients		Ethnic Groups		
		Male	Female	Malay	Chinese	Indian
Normocyte	31.5 (7-58)	19	11	19	5	6
Elliptocyte	32.0 (1-79)	16	14	30	0	0
Spherocyte	26.0 (18-46)	0	8	1	6	1

TABLE 2

PERCENTAGE LYSIS (MEAN VALUES) OF OSMOTIC FRAGILITY TEST

Nacl (%)	0.85	0.75	0.65	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20
N	0	0	0	0	0	2.1	18.1	66.1	88.6	100	100	100
SE	0	0	0	0	0	0.2	1.3	0.6	0.6	0	0	0
E	0	0.4	1.6	3.0	7.7	19.3	39.2	66.5	87.0	96.0	99.0	100
SE	0	0	0.2	0.2	1.5	2.1	1.9	1.5	0.6	0.4	0.2	0
S	0	13.8	34.3	49.9	64.3	78.0	90.4	98.7	99.7	99.9	100	100
SE	0	0.9	1.9	1.9	1.5	1.2	0.6	0.4	0.2	0	0	0

N = Normocytes; E = Elliptocytes; S = Spherocytes; SE = Standard Error

TABLE 3

GLYCEROL LYSIS-TIME TEST

The results were expressed as the time required for the absorbance to fall to half the initial value (GLTSO).

Group	Number of Samples	Mean GLTSO (min)	SE (min)
Normocyte	30	23.4	0.13
Elliptocyte	30	11.4	0.11
Spherocyte	8	6.6	0.59

SE = Standard Error

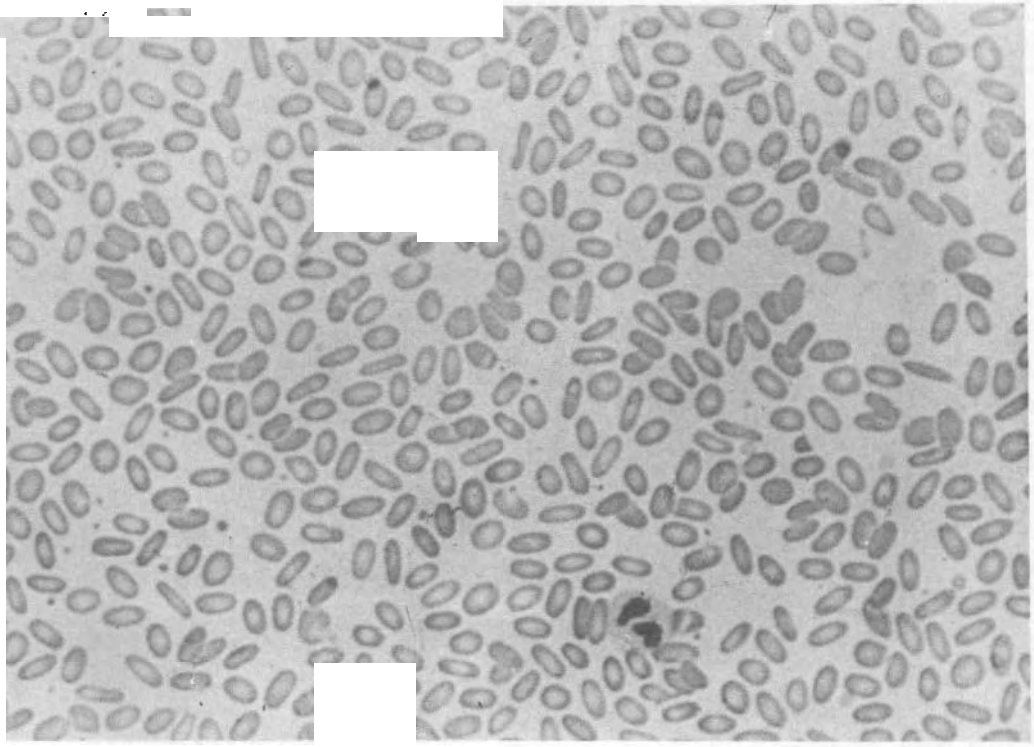

FIG 1 : Common hereditary elliptocytosis. Wright x 400.

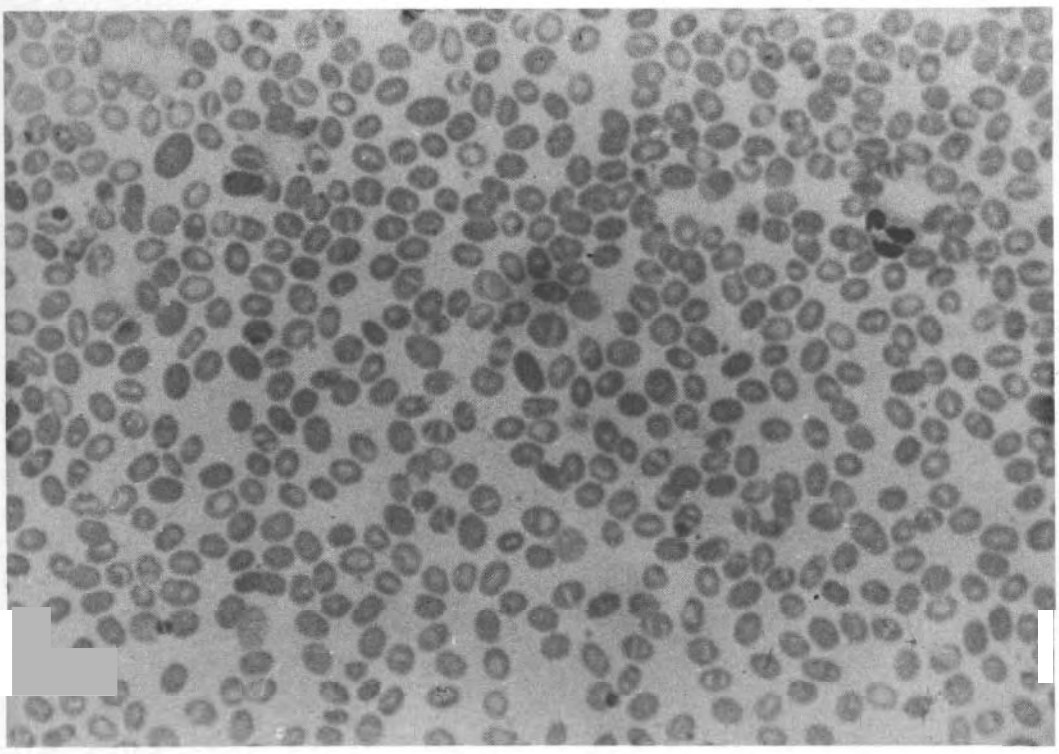

FIG 2 : Hereditary stomatocytic elliptocytosis. Wright x 400.

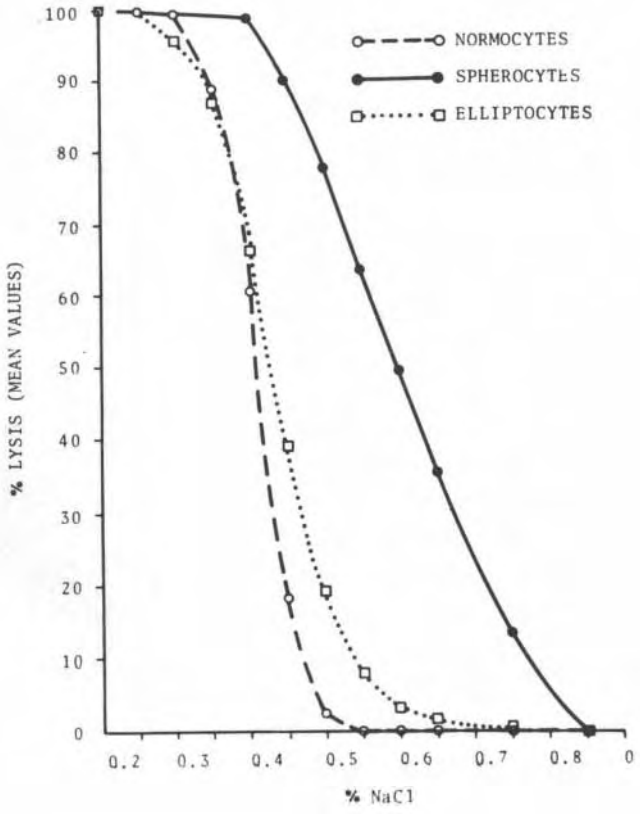

FIG. 3: Osmotic fragility curves of normocytes, elliptocytes, and spherocytes.

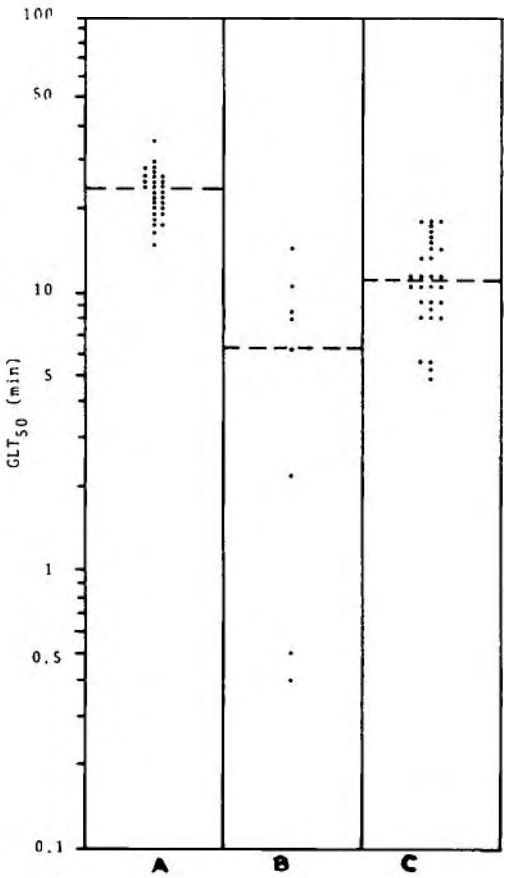

FIG. **4:** Distribution of GLT50 of normocytes (A), spherocytes (B), and elliptocytes (C).

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