HAEMOGLOBIN E — β THALASSAEMIA REEXAMINED

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SYNOPSIS

83 Malays with HbE β thalassaemia who were not transfusion dependant were investigated. 79 persons showed no β^A formation indicating the predominant gene in Malays with HbE β thalassaemia was β°. HbF assays showed levels that were similar to transfusion dependant patients. Further studies are necessary to determine the presence of the α_2 ($\alpha+$) gene interacting with HbE and β° to produce the milder phenotype of HbE β thalassaemia.

SING MED J. 1988; 29:45–47

INTRODUCTION

Haemoglobin E (HbE) is very common in South-East Asia where the trait reaches a frequency of almost 50% in the population in many areas (1). HbE is a structural haemoglobin variant that results from the substitution of glutamic acid by lysine at position 26 in the chain. In a micromapping study of 916 Malay males in West Malaysia, HbE was found in 4% and β thalassaemia in 2% (2).

In 1954 the first description of HbE β thalassaemia appeared under the title of Mediterranean anaemia; a study of 32 cases in Thailand (3). The first case found in Malaya was mentioned in a report by Lehmann and Singh (4).

HbE β thalassaemia poses a major health problem. Although the disease has been in existence for over 20 years it is far from clear why the interaction of HbE and β thalassaemia produces a severe clinical disorder not unlike transfusion dependant homozygous in some persons, whereas in others it produces clinical features of thalassaemia intermedia. This milder form of HbE β thalassaemia reflects the presence of a β+ gene, α 2 gene or an activation of Haemoglobin F (HbF) Synthesis (2). The present study was undertaken to investigate these possibilities in 83 Malays with HbE β thalassaemia who were not transfusion dependant.

MATERIALS AND METHODS

A total of 83 Malays with HbE β thalassaemia who were not transfusion dependant were studied.

The youngest was 2 and the oldest 55 years old.

Ten millilitres of blood was taken from each subject by venepuncture. An aliquot of 4.5ml in EDTA was used to determine the haemoglobin and red cell indices using a Coulter S automated counter. Routine haemoglobin analysis consisted of electrophoresis on cellulose acetate Tris-EDTA boric acid pH 8.5 and phosphate buffer pH 6.0.

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Homemade haemolysate consisting of HbA A_2 (adult blood) and HbF (cord blood) were used routinely as controls. HbA_2 and other haemoglobins were quantitated (5). HbF levels were estimated by the alkali resistant method and its distribution in red cells by the acid elution cytochemical test (6). Globin chains are separated and read by a method modified from Ueda and Schneider (7).

Osmotic fragility, sickle cell and solubility tests, intra-erythrocytic inclusions by incubation with brilliant cresyl blue were procedures included (6).

The assay for serum ferritin by an enzyme linked method was based upon the method of Addison et al (8) as modified by Miles et al (9).

Haemoglobin E was diagnosed as described by George and Sivagengei (10).

TABLE 1
HAEMATOLOGICAL MANIFESTATION OF HbE β THALASSAEMIA (NON TRANSFUSION DEPENDANT)

Hb gm/dl	7.72 (7.36 – 8.08)
RBC (x 10^{12}/litre)	3.81 (3.55 – 4.07)
MCV (fl)	68.15 (65.71 – 70.59)
MCH (pg)	21.24 (20.63 – 21.85)
MCHC %	31.10 (30.72 – 31.48)
HCt (1)	0.23 (0.23 – 24.00)
Ret (%)	2.82 (2.52 – 3.12)
HbE (%)	31.00 (30.78 – 36.42)
HbF (%)	40.70 (35.86 – 45.5)

Normal = MCV > 80fl; MCH = 28.9pg;
MCHC = 32–36gm%; HbF = 2.2%

FINDINGS

Patients with HbE β thalassaemia who were not transfusion dependant showed mild to moderate skeletal deformities with typical thalassaemic changes. Retardation of growth was mild in terms of height and weight. There was pallor of skin and mucous membranes and the conjunctiva showed a mild degree of icterus. The liver was enlarged to about 4 to 5cm and the spleen to 5cm below the costal margin; in 15 the spleen descended below the umbilicus. Massive splenomegaly resulting in physical problems due to a large spleen descending into the pelvis was seen in 4. Splenectomies done in 2 of the latter group showed no overall improvement of the haemoglobin. Marked lympha-

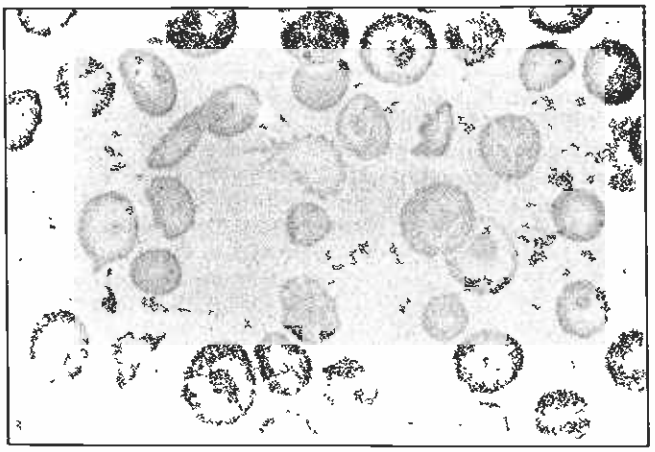

Fig. 1 The morphological appearance of red cells in non transfusion dependant HbE β thalassaemia. Typical thalassaemic cells with gross variation in shape and size, hypochromia and target cells were seen.

denopathy, aplastic crisis and megaloblastic anaemia were not significant factors. The liver and renal profiles were normal limits.

Assay for serum ferritin showed a mean of 230ng/ml (normal range 10–180ng/ml). Follow up studies done 2 years later of the two cases of HbE β who underwent splenectomy showed a rise of serum ferritin levels to 540 and 620ng/ml respectively.

The anaemia was of a moderate degree with the mean haemoglobin at 7.72gm/dl. The red cell indices were similar to those in homozygous β thalassaemia. The red cell morphology showed marked anisopoikilocytosis, moderate hypochromia and microcytosis as illustrated in figure 1. Target cells formed 15 to 25% of the red cells on the stained blood film with increased numbers of target cells and nucleated red cells seen with splenectomy. Platelet and white cells were normal.

Haemoglobin consists of E, F, A_2, and haemoglobin A (α_2 β_2) was absent in 79 persons as illustrated in Figure
2.

The mean HbF was 40.7% and this was heterogenously distributed amongst the red cells.

DISCUSSION

Most cases of HbE β thalassaemia in South East Asia and India are due to the interaction of ^{O} thalassaemia with HbE (1). In this study the predominant gene in Malays with HbE β thalassaemia who were not transfusion dependant was β^{O} showing no HbA ($\alpha_2\beta_2$) formation. The milder form of HbE β thalassaemia did not reflect the presence of the $\beta+$ gene which was seen in only 4 persons.

In transfusion dependant HbE β thalassaemia the range of HbF seen is 5 to 85% (11). The haemoglobin F mean level was 40.7% (35.8 – 45.5) ruling out the protective effect of the activation of HbF synthesis.

In our studies the nature of the milder form of HbE β thalassaemia could not be ascertained. Gene mapping studies are not available in this region and the interaction with an α_2 ($\alpha+$) gene remains to be studied.

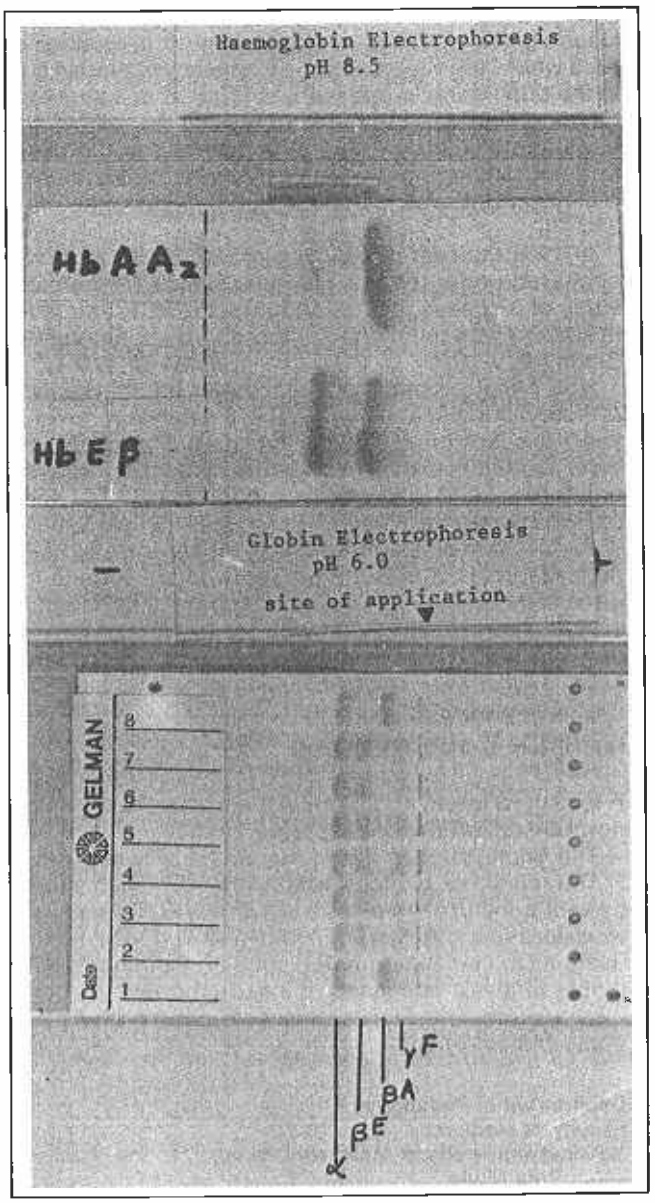

Fig. 2 HbE β^{O} thalassaemia. Electrophoretic mobilities of haemoglobin at pH 8.5 and globin chains in urea TEB citrate buffer pH 6.0

Channel 1 and 8 are normal specimens. In channels 2 to 7 are non transfusion dependant cases HbE β thalassaemia.

ACKNOWLEDGEMENT

The authors would like to express gratitude to Puan Putri Noor Aziyah for typing the manuscript.

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