bjh review

Studies in haemoglobin E beta-thalassaemia

Nancy F. Olivieri,[1] Giulia M. Muraca,[1] Angela O'Donnell,[3] Anuja Premawardhena,[3] Christopher Fisher[3] and David J. Weatherall[3]

[1]Hemoglobinopathy Research Program, Toronto General Hospital, University of Toronto, Toronto, ON, Canada, [2]MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK, and [3]Department of Medicine, University of Kelaniya, Kelaniya, Sri Lanka

Summary

Haemoglobin E β-thalassaemia is the commonest form of severe thalassaemia in many Asian countries, but little is known about its natural history, the reasons for its clinical diversity, or its optimal management. Despite its frequency, haemoglobin E β-thalassaemia is often managed in an ill-defined and haphazard way, usually by demand transfusion. We studied a cohort of Sri Lankan patients with haemoglobin E β-thalassaemia over 5 years, and identified several genetic and environmental factors possibly contributing to the phenotypic diversity of the disorder. These included modifiers of haemoglobin F production, malaria and age-related changes in adaptation to anaemia. Our findings suggest that in many patients, haemoglobin E β-thalassaemia can be managed without transfusion, even with low haemoglobin levels. Age-related changes in the pattern of adaptation to anaemia suggest that more cost-effective approaches to management should be explored.

Keywords: thalassaemia, haemoglobin E, natural history, phenotypic diversity, genetic modifiers.

The thalassaemias are a family of inherited disorders of globin synthesis, of which the most important are the β-thalassaemias (Weatherall & Clegg, 2001a). Extremely diverse phenotypes exist within the homozygous and compound heterozygote states for beta-thalassaemia. At the severe end of the spectrum are patients whose clinical course is characterized by profound anaemia, who present to medical attention in the first year of life, and who subsequently require regular transfusions for survival – the condition known as beta-thalassaemia *major*. But many patients with inheritance of two mutant beta alleles have a milder illness, with a broad range of severity including, at least in early childhood, a virtually asymptomatic state. Patients in this group who present to medical attention in later childhood and remain largely transfusion-free are said to have thalassaemia *intermedia*. The terms thalassaemia 'major' and 'intermedia' lack specific molecular correlates, but encompass a wide spectrum of clinical, as well as laboratory abnormalities (Cao, 1988; Camaschella & Cappellini, 1995; Rund *et al*, 1997; Ho *et al*, 1998; Weatherall & Clegg, 2001a).

Correspondence: Nancy F. Olivieri, MD, FRCPC, Toronto General Hospital, 200 Elizabeth Street, Eaton Wing North, Room 226, Toronto, ON Canada M5G 2C4. E-mail: noliv@attglobal.net; nolivi@gmail.com

The global importance of thalassaemia 'intermedia': haemoglobin E thalassaemia

Globally, the intermediate forms of beta-thalassaemia do not cause a major public health problem (Olivieri, 1999), except for the case of haemoglobin E beta-thalassaemia (Weatherall *et al*, 1985). Worldwide, haemoglobin E thalassaemia is one of the most important varieties of thalassaemia (World Health Organization (WHO), 1983; Chen, 1996; Weatherall & Clegg, 2001b). The condition results from co-inheritance of a beta-thalassaemia allele from one parent, and the structural variant haemoglobin E from the other (Weatherall, 1965). Haemoglobin E results from a G→A substitution in beta codon # 26 which, as well as producing a structurally abnormal haemoglobin, also activates a cryptic splice site that causes abnormal messenger RNA processing; because the usual donor site has to compete with this new site, the level of normally spliced, that is beta^{E}, mRNA is reduced (Orkin *et al*, 1982). The abnormally spliced mRNA is non-functional because a new stop codon is generated. As a result haemoglobin E is synthesized at a reduced rate and behaves like a mild form of beta-thalassaemia.

Because of the extremely high frequency of this variant within the Indian subcontinent and Asia, including carrier frequencies of up to 60% in parts of North East Thailand and Cambodia, it is very common for individuals in this region to inherit both haemoglobin E and beta-thalassaemia. Data collected over recent years indicate that haemoglobin E beta-thalassaemia is causing an increasingly severe public health problem throughout the Indian subcontinent and parts of Southeast Asia. In Thailand, it is estimated that nearly 3000 children are born with this condition each year and that there are approximately 100 000 affected patients in the population (Flint *et al*, 1998). Similarly, it accounts for nearly half of the cases of severe beta-thalassaemia in Indonesia, and for about one-third of those in Sri Lanka. Haemoglobin E is also seen

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with increasingly frequency in immigrant populations from these regions, both in the USA and Europe (Rees *et al*, 1998a).

Despite its frequency, haemoglobin E beta-thalassaemia is often managed in an ill-defined and haphazard way, usually by demand transfusion. A survey in the UK found that the only correlation with the use of regular transfusion was the patient's country of birth (Rees *et al*, 1998a). Very little is known about its clinical course of the fate of older patients who have survived childhood.

Prospective studies of haemoglobin E thalassaemia in Sri Lanka

Over recent years, increasing numbers of patients with severe forms of thalassaemia have been presenting to medical attention in Sri Lanka, where the disease is emerging as an increasingly important public health problem. Sri Lanka, a tropical island of approximately 19 million people, has a population comprised of Sinhalese (74%), Tamils (18%), Moors (7%) and several minor groups including a small number of the original island occupants, the Veddahs. Since 1951, there have been sporadic reports of the occurrence of thalassaemia and haemoglobin variants, including haemoglobin E, in most populations (de Silva & Weeratunge, 1951; Graff *et al*, 1954; Lehmann, 1956; Nagaratnam *et al*, 1958; de Silva *et al*, 1962; Nagaratnam & Sukumaran, 1967; Parameshwaran, 1967; Blackwell *et al*, 1974; Ellepola *et al*, 1980; Nagaratnam, 1989). In 1996, we began to assess the potential future health burden that thalassaemia might pose for Sri Lanka, and to attempt to better understand the natural history and clinical heterogeneity of haemoglobin E thalassaemia in this population. To calculate the burden that thalassaemia may place on the island's health services in the future, we began by carrying out a pilot screening programme to determine its approximate gene frequencies. In 1600 schoolchildren and 703 patients with beta-thalassaemia, 23 different β-thalassaemia mutations, with three accounting for the thalassaemia phenotype in approximately 70% of patients, were identified (De Silva *et al*, 2000). Interactions of two common alleles would be expected to usually result in a transfusion-dependent disorder. The third common mutation, which produces haemoglobin E, interacts to produce haemoglobin E thalassaemia.

Some observations in this study were critical future in consideration for the planning for control of the thalassaemias, particularly in the large rural populations of South East Asia. We observed that even a low frequency of haemoglobin E in a population with thalassaemia had an important effect on the overall burden of disease. We calculated that patients with haemoglobin E β-thalassaemia would be expected to account for 40% of approximately 2000 thalassaemia patients expected to require treatment at any one time in the future in Sri Lanka. However, based on the Hardy–Weinberg distribution, we should have encountered many more cases of haemoglobin E thalassaemia, probably reflecting the mildness of some cases of haemoglobin E and its variable clinical course. Finally we observed that, even on a small island, there was a wide difference in the frequency of mutations between regions, emphasizing the importance of widespread sampling to assess approximate gene frequencies of thalassaemia. This finding, and the fact that much of the present data on haemoglobin E thalassaemia in Asia are based on studies of limited numbers obtained years ago (WHO, 1994), suggests that careful population data are needed for future planning for control of the thalassaemias.

Clinical studies of haemoglobin E thalassaemia in Sri Lanka

While the variable clinical phenotype of haemoglobin E β-thalassaemia has been reported in several populations (Chernoff *et al*, 1956; Wasi *et al*, 1969; Fucharoen & Winichagoon, 1987; Khanh *et al*, 1990; George & Wong, 1993; Winichagoon *et al*, 1993; Agarwal *et al*, 1997; Rees *et al*, 1998b; Weatherall & Clegg, 2001a), less has been reported about its natural history, the reasons for its clinical diversity, or the most appropriate management. In 1996, investigators from Oxford and Toronto undertook a modified 'natural history' study of haemoglobin E thalassaemia in Sri Lanka. Patients with haemoglobin E thalassaemia at the Kurunegala General Hospital were studied (Table I); 31 additional patients aged 13 years or younger were assessed separately (Table II). Patients underwent clinical assessment including clinical examination at least four times each year, including measurement of height, weight, growth velocity, bone age, mid-parental height, sexual maturation, skeletal deformity and quality-of-life. When clinically indicated, liver biopsy samples were analysed for histology and hepatic iron concentration.

Cardiac ejection fractions and pulmonary artery pressures were measured by 2D transthoracic echocardiography and Doppler. Haemoglobin analysis was performed by high pressure liquid chromatography (BioRad, Hercules, CA, USA). Serum erythropoietin levels were measured in duplicate on at least three occasions using a Quantikine *In Vitro* Diagnostics enzyme-linked immunosorbent assay (ELISA) kit (R & D Systems, Abingdon, UK). DNA analysis was carried out for primary, secondary and tertiary phenotypic modifiers (Weatherall, 2001) followed standard methods; DNA was extracted and analysed for *HBA* and *HBB* gene mutations (Fisher *et al*, 2003), identification of TA repeat numbers in the *UGTA1A* promoter (Premawardhena *et al*, 2001), C→G (−/+) polymorphism at position $^{G}\gamma$-158 (Fisher *et al*, 2003) and haplotypes for the gene for α haemoglobin stabilizing proteins (Viprakasit *et al*, 2004). *Plasmodium falciparum*-specific IgG in serum was measured by ELISA (Snounou *et al*, 1999).

As of 1997, 109 patients, who varied in age from 1–51 years, were categorized as having haemoglobin E β-thalassaemia; 23% were not receiving blood transfusions, while the remainder had been maintained on regular or intermittent

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Table I. Clinical, haematological and genetic findings in patient groups with HbE/β thalassaemia.

	Patient group				
Class	1	2	3	4	5
No.	25	37	14	22	11
Age (years)	4–51 (25·3 ± 16·5)	9–52 (23·2 ± 10·6)	5–16 (10·8 ± 3·0)	5–21 (15·2 ± 3·4)	4–13 (?±2·9)
Age diagnosis (years)	1·5–4·8 (15·5 ± 13·4)	0·8–35 (9·9 ± 8·4)	0·4–56 (2·3 ± 2·1)	0·7–8·0 (3·6 ± 2·6)	0·2–4·0 (1·4 ± 0·8)
Maximum liver size (cm)	0–14 (96·0 ± 3·5)	3–18 (9·8 ± 3·5)	6–16 (9·8 ± 1·6)	5–19 (11·1 ± 3·8)	0–12 (7·6 ± 1·7)
Maximum spleen size (cm)	0–18 (8·9 ± 3·2)	6–23 (13·4 ± 3·2)	11–20 (17 ± 38)	5–17 (11·4 ± 3·3)	4–15 (9·9 ± 3·3)
Facial deformity (0–6)	0–2 (0·9 ± 0·7)	0–4 (1·5 ± 1·0)	0–4 (2·0 ± 1·2)	0·5–3·5 (1·92 ± 1·2)	1–3 (1·5 ± 0·9)
Splenectomized (%)	21·0	62·2	100	85	22
Time from splenectomy (years)	2–33 (13·3)	2–19 (7·4)	2–9 (3·1)	1–13 (5·6)	2–7 (4)
History major infection (%)	8·3	18·9	16·7	26·3	11·0
History of malaria (%)	21	37·9	25·0	42·0	11·0
Leg ulcers (%)	33·0	51·3	16·7	15·8	0
Fractures (%)	0	13·9	0	25·0	56
Gallstones (%)	22·0	41·7	9·1	16·7	0
Cholecystectomy (%)	13·0	5·4	0	5·3	0
Hb (g/l)	31–82 (65 ± 12)	36–83 (61 ± 10)	42–71 (58 ± 10)	44–80 (59 ± 12)	40–58 (47 ± 7.0)
Presplenectomy	–	–	3·5–4·3 (3·9)	–	–
Postsplenectomy	6·5–7·6 (7·0)	5·3–7·2 (6·4)	6·4–8·7 (7·2)	4·1–7·6 (6·1)	4·0–5·5 (4·9±?)
Platelets (10^9/l)					
Presplenectomy	157–898 (400·5)	223–749 (432)	203–576 (365)	294–474 (392)	287–503 (364·5)
Postsplenectomy	379–918 (742)	253–1051 (677)	411–948 (690)	303–974 (679)	335–814 (574)
Hb F (g/l)	5·0–38 (20 ± 12)	2·0–37 (17 ± 7·0)	5·0–25 14 ± 5·0)	4·0–27 (15 ± 3·0)	4·0–19 (9·0 ± 4·0)
Hb F (%)	8–60 (32 ± 13)	3–48 (29 ± 10)	10–34 (20 ± 6)	10–42 (26 ± 11)	6–42 (17 ± 8)
β-thalassaemia mutation (%)					
IVS-1-5 (G→C)	52·0	73·5	74·6	59·1	75·0
IVS-1-1 (G→A)	39·0	26·5	27·3	22·7	13·0
Others	9·0	0	8·1	18·8	12·0
α^+-thalassaemia (%)	8·0	8·1	7·1	4·5	6
ααα/αα (%)	4·7	3·0	0	0	0
Xmn 1 +/+ (No)	8	5	0	0	0
Xmn 1 −/− (No)	2	2	2	5	4
ALT (U/l)					
HIC (mgFe/g/100 dry weightt)	2·5–26 (9·3)	2·7–42 (14·8)	5·4–52 (16·7)	4·7–30 (15·0)	4·2–28 (16·3)

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('on demand') transfusion. It was not clear why these patients were receiving transfusions; their pretransfusion haemoglobin levels (mean 70 g/l) showed little difference from those who were not (mean 61 g/l). As judged by initial serum ferritin or hepatic iron concentrations, many transfused patients already had dangerously high body iron burdens (Olivieri & Brittenham, 1997). Accordingly, it was decided to stop transfusion and observe the patients closely; progress was followed from 1997 to the present day. Folic acid and subcutaneous deferoxamine were prescribed when indicated; splenectomized patients were prescribed prophylactic penicillin, and instructed to report with symptoms of infection.

Because of the patients' wide age range, clinical diversity, changing phenotypes, and variation in management before the study, for descriptive and analytical purposes their clinical status was classified into five groups, defined in Table III. Largely due to rapidly changing phenotypes in some younger patients, 10 changes in categorization were made during the final period of observation (Premawardhena *et al*, 2005).

Complications

Hypersplenism

Splenomegaly was much more marked in severe phenotypes, groups 3–5. In group 3 patients, splenectomy resulted in an improvement in growth and development, and in a significant rise in haemoglobin concentration (Table I). Since these children were only observed for a mean of 2 years, it is not yet clear whether improvement will be sustained; comparing the steady-state haemoglobin values of those who had been splenectomized with those of the total groups (Table I) it seems unlikely that the improvement in haemoglobin concentration will be maintained over longer periods.

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Table II. Main clinical, haematological and genetic findings in patients aged less than 13 years.

Class	1	2	3	4	5
No.	16	3	10	3	14
Age (years)	2–13 (7·1)	11–12 (11·6)	7–13 (10·7)	7–13 (11·0)	2–12 (7·1)
Age diagnosis (years)	1–9·3 (4·5)	1·7–3 (2·4)	0·8–5·6 (2·6)	1·5–3·8 (2·3)	0·2–7·0 (2·2)
Maximum liver size (cm)	0–4 (1·4)	3–6 (4·6)	4–9 (6·6)	4–7 (5·3)	2–11 (5·2)
Maximum spleen size (cm)	0–7 (3·0)	2–10 (5·6)	12–20 (17·5)	10–13 (11·0)	6–19 (12·8)
Splenectomy (*n*)	0	2	10	2	2
Hb (g/l)	46–80 (65)	61–76 (9·0)	–	30–58 (43)	33–53 (41)
Presplenectomy	–	–	3·5–4·4 (4·0)	–	–
Postsplenectomy	–	–	5·3–8·9 (7·2)	–	–
Hb F (g/l)	7·0–38 (19)	8·0–22 (16)	7·0–15 (9·0)	8·0–12 (11)	7·0–14 (11)
β-thalassaemia mutation (%)					
IVS-1-5 (G→C)	8	3	8	1	11
IVS-1-1 (G→A)	3	0	2	2	1
Others	5	0	0	0	2
α^+ thalassaemia (No)	2	1	0	0	0
Xmn 1 +/+ (No)	1	0	0	0	0
Xmn 1 −/− (No)	2	0	3	1	6
Plasmodium falciparum, IgG ELISA/IFAT (+/−)	1/9	1/0	4/4	1/0	6/3
P. vivax, IFAT (+/−)	7/5	2/0	10/0	1/0	8/3

ELISA, enzyme-linked immunosorbent assay; IFAT, immunofluorescent antibody test.
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Table III. Descriptive classification of patients with HbE/β thalassaemia.

Group	Clinical definition
1	Minimal transfusion >20 years, 0–20 units <20 years, 0–10 units Adults: Sexually mature Children: >3rd centile height or height velocity >4 cm/year QOL >5
2	As for Class 1, except for Transfusion History Aged >20 years: ≥21 units Aged <20 years: ≥11 units
3	Postsplenectomy patients over 2 years of age: Improved QOL by at least 3 points Increase in growth >25%: meaning that a > 25% increase in *height velocity* needed to be observed over the 2-year period following splenectomy*; Improved height velocity to >3 cm/year†
4	Growth <3 centile for Ht QOL <5
5	Unable to function without transfusion

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QOL, quality of life scale, 0–10.
*If, for example, if presplenectomy height velocity during the year prior to splenectomy was 4 cm/year, a >25% increase would be represented by an increase to a height velocity exceeding 5 cm/year.
†If the patient's presplenectomy height velocity was less than 3 cm/year, the patient was considered to have improvement in growth if his or her height velocity increased to >3 cm/year.

Iron loading

The higher mean liver iron concentrations in groups 2–5 (Table I) reflect the results of long-term transfusion and inadequate chelation therapy. However, iron loading was also observed in group 1 patients who had received few transfusions (Olivieri *et al*, 2000). Of 15 patients in this group who underwent hepatic biopsy, half of those aged 20 years or older had hepatic irons exceeding 10 mg/g dry weight. There was an associated increase in hepatic fibrosis and in one case, frank cirrhosis (Olivieri *et al*, 2000).

Bacterial and viral infection

Severe life-threatening infections included pyogenic abcesses in spleen, brain, liver and kidney, and severe respiratory tract infections; in all but one patient, these episodes occurred in splenectomized patients. At the beginning of the survey, only one patient showed positive serology for hepatitis C and one for hepatitis B; this had not changed at the end of the observation period. Serology for human immunodeficiency virus (HIV) was negative in all patients. The low prevalence of viral infection in these regularly transfused patients is probably the result of routine screening of blood for these viruses in laboratories servicing this area.

Malaria

A history of malaria unconfirmed by blood film examination, was obtained from patients in each group. Sera analysed for IgG

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antibodies to *P. falciparum* and *P. vivax* to determine whether patients had been previously infected revealed that 57% of patients had positive serology for *P. falciparum*; 79% were positive for *P. vivax*. An analysis of the children under the age of 13 years indicated that those in the severely affected groups who developed early splenomegaly and anaemia showed a trend to positive serology for *P. falciparum*, but not *P. vivax* ($P \leq 0{\cdot}034$).

Other complications

The higher frequency of leg ulcers and gallstones in groups 1 and 2 probably reflected their higher mean age (Table I). Fractures were unusual; in only one patient had a fracture occurred without significant trauma. Similarly, severe facial deformity was observed in only two patients. Although persistent thrombocytosis after splenectomy was common, there were no thrombotic episodes. Twelve patients, of whom 10 had been splenectomized, were found to have mildly elevated pulmonary artery pressures. One group 1 patient developed spinal cord compression due to extramedullary haemopoiesis; a full recovery followed hydroxycarbamide and transfusion therapy.

Deaths

There were eight deaths over the period of observation, four due to infection: postoperative empyema (in a patient aged 51 years), severe biliary tract infection (in a patient aged 32 years), occipital-lobe abcess (in a patient aged 23 years) and pyogenic meningitis (in a patient aged 11 years). Four patients aged 17–42 years died from iron loading of the liver and myocardium.

Mechanisms of clinical diversity in patients with haemoglobin E thalassaemia

Anaemia

The mean steady-state haemoglobin level in the milder groups was $63 \pm 1{\cdot}4$ g/l; although statistically different (at $55 \pm 1{\cdot}8$ g/l; $P < 0{\cdot}0001$) from that in the severe groups (3–5), this was not clinically different.

β-thalassaemia mutations

The two common Sri Lankan mutations [IVS1-5 (G→C) and IVS1-1 (G→A)] are β° and severe β^{+}-thalassaemia mutations, respectively; only four other mutations were identified in this group. Hence, none of the phenotypic heterogeneity was ascribed to primary modifiers, i.e. mild β-thalassaemia mutations.

Haemoglobin F

Much of the difference in haemoglobin values between the groups reflected the ability to produce haemoglobin F. There was a correlation within the narrow range of haemoglobin values between the haemoglobin concentration, and the absolute concentration of haemoglobin F ($P \leq 0{\cdot}001$). Haemoglobin F values showed a strong correlation with the *Xmn*-I polymorphism –158 to *HBG2* ($^{G}\gamma$ gene); the 13 individuals with the genotype ++ were restricted to groups 1 and 2, and their mean age was 33 years (compared with 19 years for the entire study group). There was a paucity of the ++ genotype, and an over-representation of the −/− genotype, in young children with haemoglobin E β-thalassaemia.

α-globin genes

The frequence of alpha-thalassaemia ($-\alpha/\alpha\alpha$) in the Sri Lankan population is approximately 14%. The restriction of α^{+}-thalassaemia alleles to the milder groups 1 and 2, and a lower frequency in patients with haemoglobin E thalassaemia (2·3%) compared with those with β-thalassaemia major (7·6%), suggests that α-thalassaemia is under-represented in this hospital population of patients with haemoglobin E thalassaemia. This raises the likelihood of a significant ameliorating effect of alpha-thalassaemia on the severity of haemoglobin E thalassaemia. There was no difference in the distribution of haplotypes of the gene for AHSP (*ERAF*) between patients with the mild and severe phenotypes.

Jaundice

There were significantly higher bilirubin levels in those with the 7/7 genotype of the *UGTA1A* promoter, compared with those with 6/6 and 6/7 genotypes ($P = 0{\cdot}032$ and $0{\cdot}0015$ respectively). Those with the 7/7 genotype were more likely to develop gallstones over the age of 15 years ($P \geq 0{\cdot}0008$). Preliminary details of these findings have been reported previously (Premawardhena *et al*, 2001).

Response to anaemia

Serum erythropoietin (Epo) assays over the period of observation demonstrated a strong correlation between Epo and haemoglobin concentrations ($P \leq 0{\cdot}0001$), as well as a decline in Epo response to anaemia with age ($P \leq 0{\cdot}0001$). Multiregression analysis confirmed that age, and Epo response to anaemia, were independent variables. These age-related changes were also reflected in changes in spleen size. Measurements of the spleen were compared between two age groups of group 1 and 2 patients: in the first group, 24 patients aged 15–51 years, the mean increase in spleen size was 0·05 cm/month; 10 of 24 spleens showed no increase in size. By contrast, in the second group, of 13 patients aged less than 15 years, mean increase in spleen size was double this rate, at 0·10 cm/month (O'Donnell *et al*, 2007).

Discussion

Our work highlights the problems in defining the phenotypic diversity and natural history of the thalassaemias

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(Premawardhena *et al*, 2005). Our first analysis of the frequencies of β-thalassaemia and haemoglobin E (de Silva *et al*, 2000) reported a paucity of patients with haemoglobin E thalassaemia. This suggested that the Sri Lankan hospital-based population may not be representative of the milder spectrum of haemoglobin E thalassaemia, which is not usually considered in clinical descriptions of the haemoglobinopathies. Our subsequent studies showed wide diversity in age and medical intervention and, in the younger patients, rapidly changing phenotypes. While our analysis provides a picture of the course of the disease in early life, it may also be influenced by an early presentation of severe disease. Despite these reservations, it is possible to draw some tentative conclusions about the basis for the clinical diversity of haemoglobin E thalassaemia in Sri Lanka.

Despite the genetic homogeneity of the patients, there was considerable phenotypic heterogeneity. We were surprised to find that this occurred within a relatively narrow range of haemoglobin values, with the mean difference between the mildest and most severe groups approximately 10–20 g/l.

The haemoglobin was correlated with the ability to produce haemoglobin F, which, in turn, is strongly related to the *HBG2* promoter polymorphism. The relationship between this polymorphism and the steady-state level of haemoglobin F, its association with a later age of presentation, and its increased representation in the older age groups indicate that it is a major modifier of the haemoglobin E thalassaemic phenotype in Sri Lanka.

There appeared to be a significant ameliorating effect of alpha-thalassaemia on the severity of haemoglobin E thalassaemia in Sri Lanka. A paucity of α-thalassaemia was observed previously in a hospital population with haemoglobin E thalassaemia (Winichagoon *et al*, 1985), supporting our present findings.

Similarly, the high frequency of the 7/7 allele of the *UGTA1A* promoter in Sri Lanka is related to the occurrence of deep jaundice and an increased frequency of gall bladder disease in patients with haemoglobin E thalassaemia (Premawardhena *et al*, 2001, 2003).

However, not all is so clear. While ten of 27 children with rapidly enlarging spleens had the −/− genotype at the *HBG2* promoter, this cannot be the only explanation for the severity of the disease in this group. The fact that those who underwent splenectomy restored their Hb levels, at least in the short term, to those of the milder groups indicates that other factors must be involved in defining their severe phenotype. Environmental factors, hitherto neglected in the thalassaemia field, may be of considerable importance.

Our malaria-serology data showing a high level of exposure to *P. falciparum* and *P. vivax,* and a trend towards an increased frequency of IgG antibodies to *P. falciparum*, in more severely affected young children, indicate that further studies to analyse the interaction between malaria and haemoglobin E thalassaemia are urgently required, particularly in view of the resurgence of malaria in many parts of Asia.

The wide variation in erythropoietin response to anaemia, and its decline with age, was unexpected. Although a similar trend was observed in a small group of patients with sickle cell anaemia (Sherwood *et al*, 1986), variation in adaptation to anaemia at different ages has not been documented in the haemoglobinopathies. The strong direct correlation between concentrations of serum erythropoietin and serum transferrin receptor indicates that profound elevations in erythropoietin are relevant to marked erythroid expansion. The observation that erythropoietin levels were lower in our older patients may have important implications for the treatment of haemoglobin E thalassaemia at different stages of life. The rate of splenic enlargement at particular haemoglobin levels also declined with age although, as yet, there is no direct evidence that the mechanisms are related.

Clinically, the patients who constitute groups 1 and 2 have a relatively mild disease characterized, among other clinical findings, by adequate growth and sexual development. The facility of these patients to adapt to anaemia more effectively than those with other forms of thalassaemia 'intermedia' may reflect the relatively low levels of haemoglobin F combined with the properties of haemoglobin E on the oxygen dissociation curve, and hence on oxygen delivery. Further work is required to assess potential risks in later life for such patients who have survived for many years at relatively low haemoglobin levels.

Some severely affected children showed a marked improvement in growth and development, though a less impressive rise in the haemoglobin level, after splenectomy. In parallel there was a high frequency of infection in splenectomized patients that was not restricted to early life. Other postsplenectomy complications appeared less clear. Unlike reports from Thailand (Wasi *et al*, 1982; Sonakul & Fucharoen, 1992), we did not observe right heart failure associated with postsplenectomy thrombocytosis, although slightly raised pulmonary artery pressures were found in 10 cases.

What have we learned?

What are the practical implications of these observations? First, it is clear that no patient with haemoglobin E thalassaemia should receive regular transfusion without a long period of observation of growth and development, quality of life, and spleen size. Because of the facility to adapt to low haemoglobin levels, the haemoglobin *per se* is of limited value in deciding on transfusion. The age of presentation, *HBG2* promoter polymorphism and, probably, co-existent α-thalassaemia, are valuable prognostic indicators.

Several important questions remain. First, while some information has been obtained about the clinical course and reasons for the phenotypic heterogeneity of haemoglobin E thalassaemia, a cross-sectional study of different ages of this type suffers from the same difficulties as many reports of studies of the intermediate forms of thalassaemia. In particular, because of rapidly changing phenotypes, variable medical

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intervention, and the ill-understood effects of age on phenotype, it is difficult to accurately characterize disease severity. And although some genetic factors have been defined as possible modifiers, it is clear that we still incompletely understand the remarkable phenotypic diversity of this condition. Such understanding would be augmented by a cohort study from birth or, because a significant proportion of patients with this condition present later than the first year of life, in a group of children under the age of 12 years whose phenotypes are defined as clearly as possible before any form of medical intervention.

Given what we have learned, what is the optimal management of patients with haemoglobin E thalassaemia who fail to adapt to anaemia? Although in this series we observed a high frequency of splenectomy, and some evidence for its beneficial effect on growth and development, it is not clear whether the frequent use of splenectomy is acceptable. Even in our relatively small series, morbidity and mortality due to infection at all ages was significantly higher in splenectomized patients. Since it is now clear that it is possible to stop transfusions in this disorder, and it appears that marrow expansion and increasing splenomegaly are less marked in older patients, a trial or observational study of 'transient transfusion' during the period of major expansion seems justified (see *Appendix I*).

What about the management of other complications? We also have established that there is a variable rate of iron loading from the gastrointestinal tract in minimally transfused patients; it is clear that some such patients are at risk for hepatic fibrosis and other complications of iron loading. It will be important to establish the reasons for variable iron loading. Next, there is a small but important group of patients who appear to have problems of growth and sexual retardation during their pubertal years, in the absence of severe iron loading. The mechanisms require further exploration; some insight may be gained from preliminary studies of individual responses to anaemia, a subject neglected in the thalassaemia field. There still are limited data on the environmental factors that might modify the course of this disease. In emerging countries, the recrudescence of malaria may be an important factor; little is known about the susceptibility of patients with different forms of thalassaemia intermedia, with or without intact spleens, to this important infection (Weatherall & Clegg, 2002). Data of this type are urgently required.

Finally, our earlier screening studies suggest that while further screening would achieve better precision in estimating future health burden, it is already clear that management of these disorders for example could require about 5% of the total health budget of Sri Lanka. This will be similar to many Asian countries where improved living conditions and public-health measures have caused a major decrease in childhood mortality rates (Weatherall & Clegg, 1996; Weatherall, 2005). The practical implications are clear: the costs of thalassaemia care will represent an important proportion of the healthcare budgets of Asia (Weatherall & Kwiatkowski, 2002). Hence, consideration of general guidelines for management, which include careful consideration of maintaining such patients off transfusions, have been suggested for the management of haemoglobin E thalassaemia (*Appendix I*).

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Appendix I: Recommendations for management of haemoglobin E thalassaemia

Diagnosis, including Initial Assessment

1. The diagnosis of Hb E thalassaemia is easily made in the non-transfused state by haemoglobin electrophoresis or by high performance liquid chromatography (HPLC). Even if the patient has been transfused, and/or HPLC is not available, the presence of haemoglobin E on haemoglobin electrophoresis raises the possibility of this diagnosis.

2. Genotyping of the parents often helps to confirm the diagnosis. Screening of available siblings (who may be asymptomatic, especially if the index case has a late clinical presentation) is recommended.

3. Whenever possible, DNA sample should be obtained and stored. The presence of alpha thalassaemia and the polymorphism T to C -158 5' to *HBG2* (Xmn 1) should be determined. While not fully explaining the diversity of phenotypes, both alpha thalassaemia and the *Xmn 1* polymorphism appear to have a large effect on reducing disease severity.

4. Age at presentation may be a useful prognostic indicator; if a patient presents after the age of 4 or 5 years, it is unlikely that he or she will immediately require regular transfusions.

5. The newly diagnosed patient with haemoglobin E thalassaemia should not be transfused immediately upon diagnosis. Instead, each patient should be carefully observed. A transfusion should be administered if the haemoglobin concentration is <40 g/l and/or or if the patient is suspected of having an acute intermittent infection, and/or is showing any clinical problems thought to be related to anaemia. However, even if a few transfusions have been administered in the acute situation (i.e. by other physicians on the medical or pediatric ward, for example), *immediate commitment to a transfusion program should not be undertaken, except in unusual circumstances.* In the usual circumstance, it is worthwhile to attempt to evaluate the patient in the non-emergency situation from the untransfused baseline: that is, to withdraw transfusions and carefully observe, as outlined below.

6. *During the initial assessment period* (that is, while the patient is not in the steady state) the patient should be observed every two to three weeks, ideally by the same physician. At each clinic visit, a careful history (with emphasis on the patient's appetite, energy, mood, quality of life and developmental milestones) should be recorded. A careful physical examination at each visit should record spleen and liver size, height, and weight.

7. *During the initial assessment period,* when the decision to administer regular transfusions is not yet reached, it is especially important that a full blood count be obtained on the two- to three-weekly visit. This should be recorded in a hospital record that can be retained by the physician for future decision-making (often patient charts provided to patients are misplaced, and an accurate history of the history and severity of anaemia is lost). Note that, in many cases, steady-state haemoglobin concentration, though significantly lower in severe phenotypes than in milder phenotypes, is of limited value in the assessing severity of Hb E thalassaemia, including the need for regular transfusions.

Monitoring, including making the decision to transfuse

8. After 3–6 months of careful observation as above, a clinical and laboratory pattern should begin to emerge. Transfusions are unlikely to be necessary if the patient maintains a reasonable appetite, level of energy, quality of life, growth velocity (height is more indicative of growth pattern than weight), sexual maturation in parallel with bone age, and if the spleen size is stable, enlarging at a rate not greater than 3 cm/year. Regular transfusions should be considered if the haemoglobin declines below 50 g/l, or if appetite, energy, growth, or developmental milestones are compromised, or if the spleen appears to be enlarging at a rate exceeding 3 cm/year.

9. Recurrent fever, or a rapidly enlarging spleen, is an indication for thick and thin blood-film for *P. falciparum* and *P. vivax* parasites and for appropriate treatment. This should be considered before committing the patient to a long-term transfusion program.

10. There is a wide variation in the erythropoiein response to anaemia in haemoglobin E thalassaemia, such that some patients demonstrate marked marrow and organ expansion during maintenance of steady-state haemoglobin concentrations that are well tolerated (without evidence of marked marrow expansion) in others. Similarly, some patients grow poorly at concentrations of haemoglobin that are not associated with significant growth retardation in others.

11. Our studies have demonstrated a decline in the erythropoietin response to anaemia with age, and a decline in the rate of splenic enlargement at particular haemoglobin levels with age, such that progressive splenomegaly is less common after the age of 20 years. It seems logical to recommend regular transfusions for patients in whom steady-state haemoglobin levels are declining in parallel with profound enlargement of the spleen, and to continue transfusions during the period of potentially maximum response to anaemia (we suggest this may be between age 3 and 12 years) *but not to continue regular transfusions indefinitely*. The evaluation of this recommendation (i.e. a regimen of 'transient transfusion' – whereby severely affected children are transfused to maintain baseline levels of approximately 80 g/l during the period of maximum growth and development, after which transfusions are stopped – in a randomized trial, for example, would be of great interest in the management of haemoglobin E thalassaemia.

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Appendix I: (Continued)

12. Patients with Hb E thalassaemia may not require transfusion schedules that are generally applied in the case of thalassaemia 'major' arising from inheritance of thalassaemia alleles other than haemoglobin E. Our data suggest that, by contrast, a increase in haemoglobin concentration of only 10–20 g/l might have a greatly beneficial effect on the course of haemoglobin E thalassaemia. This modest goal should be more easily achievable by transfusions to maintain baseline haemoglobin concentrations of approximately 75–80 g/l. Maintenance of this range of baseline haemoglobin concentration usually results in improvement in quality of life, growth, and other parameters for which the patient has been started on transfusions.

Decisions regarding splenectomy

13. *Splenectomy versus Regular Transfusions?* When a patient with Hb E thalassaemia appears to be suffering from the effects of anaemia a dilemma often arises: whether to recommend *splenectomy* to increase the effective red cell mass pooled in an enlarged spleen, or *regular transfusions* with potential to both raise peripheral haemoglobin levels and effect a regression of extramedullary haematopoiesis (including in the spleen). There are potential advantages and drawbacks to each approach, some of which have become apparent from our studies in Sri Lanka.

14. The decision to begin regular transfusions or to recommend splenectomy in a patient with Hb E thalassaemia is often very difficult. Clearly, as with all such decisions, a comprehensive discussion with the family is recommended.

15. In our experience, many patients who undergo splenectomy appear to restore haemoglobin levels in the short term by about 10–20g/l. Some of these patients demonstrate a marked improvement in growth and development. However, the high frequency of infection in splenectomized patients does not appear to be restricted to early life and, therefore, life-long compliance with (ideally, 3-weekly intramuscular) penicillin is recommended. This may represent a disadvantage to certain patients. The potential risks of other long-term complications of splenectomy, including pulmonary hypertension and thrombosis, are unclear.

16. Presplenectomy preparation should include vaccination with 23-valent pneumococcal vaccine at least one month prior to splenectomy. Communication should be established with the surgical teams to ensure that a liver biopsy is obtained during surgery (to be picked up in an iron-free tube, stored at −20°C, and shipped promptly to a laboratory for quantitation of iron concentration).

17. Many patients with Hb E thalassaemia will not need further regular transfusions following splenectomy. Therefore, except in the acute postoperative period, patients who have undergone splenectomy should not be transfused for at least 6–12 months following splenectomy and until postsplenectomy steady-state haemoglobin concentrations are reached. This period should be managed similarly to the 'initial assessment' period described above, and regular transfusions should be re-started only if the patient cannot tolerate life off transfusions. As noted above this decision should not be determined solely by haemoglobin concentration but also by the appetite, level of energy, quality of life and linear growth of the patient.

18. In both transfused and non-transfused patients with haemoglobin E thalassaemia in the steady state (that is *after* the decision to transfuse is reached) the following evaluations should be obtained as follows:

Test	Frequency	Comment
History and physician examination	In general, every 3 months (except as above during initial assessment and following splenectomy or in patients deemed as developing an unstable phenotype) Every 6 months in other patients	Emphasis should be placed on patient's appetite, energy, mood, quality of life, developmental milestones, spleen and liver size, and growth Recording should be precise and accurate
Tanner staging	Annually after age 12 years	
Evaluation of cardiac function, including pulmonary artery pressure	Every 2 years after age 12 years	
Serum ferritin concentration (SFC)	Annually	If SFC exceeds 300 μg/l efforts should be made to assess liver iron concentration
Alanine transaminase (ALT)	Annually	More frequently if new elevation in ALT observed
Hepatitis and HIV serology	Annually	
Fasting blood glucose	Annually	
Bone age	Every 2 years after age 10 years	If significantly delayed, this may helpful in the decision to transfuse; if delayed in adolescence, suggests growth spurt still awaited; may be worthwhile to defer regular transfusions if clinically otherwise stable

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