

Haemoglobin E β thalassaemia in Sri Lanka



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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 clinical diversity, or its management. We studied 109 Sri Lankan patients with the disorder over 5 years. 25 patients were not receiving transfusion; transfusion was stopped with no deleterious effect in a further 37. We identified several genetic and environmental factors that might contribute to the phenotypic diversity of the disorder, including modifiers of haemoglobin F production, malaria, and age-related changes in adaptive function. Our findings suggest that haemoglobin E β thalassaemia can be managed without transfusion in many patients, even with low haemoglobin levels. Age-related changes in the pattern of adaptation to anaemia suggest that different and more cost-effective approaches to management should be explored.

Haemoglobin E β thalassaemia is the commonest form of severe thalassaemia in many Asian countries.^{1,2} To assess its natural history, the reasons for its clinical diversity, and how it should be managed, we studied 109 patients at the Thalassaemia Centre, Kurunegala General Hospital, Sri Lanka, between 1997 and 2003; 31 patients aged 13 years or younger who presented during the study were assessed separately. We obtained approval from the Ethics Committee of the University of Colombo, the Central Oxford Research Ethics Committee, and the Oxford Tropical Research Ethics Committee.

At least four times every year, the patients underwent full clinical examination, including assessment of quality of life (QOL; panel), height, weight, growth velocity, bone age, skeletal change, and sexual maturation. Laboratory studies included haematological and DNA analyses,^{2,4-6} multiple erythropoietin measurements (Quantikine in vitro Diagnostics ELISA kit, R&D Systems, Abingdon, UK), and screening for IgG antibodies to *Plasmodium falciparum* and *Plasmodium vivax* by ELISA, indirect fluorescent antibody test, or both, and for *P vivax* sequences by nested PCR.⁷ Summary statistics are presented with means and SD; comparisons were made with Student's *t* test or Fisher's exact test, and Pearson's correlation coefficient and multi-regression analyses were done with Stata statistical software version 8.2.

Initially, 25 patients were not receiving blood transfusions, while the remainder were maintained on regular or intermittent transfusion. Since the pre-transfusion concentrations of haemoglobin in the latter (42–86 g/L; mean 70 g/L) showed little difference from those of patients who were not receiving transfusions (38–88 g/L; mean 61 g/L) we decided to stop transfusion and observe the patients closely; details of the protocol followed between 1997 and 2002, together with criteria for the later division of patients into different phenotypic groups, are shown in the panel. Patients received folate supplementation and, when indicated, subcutaneous desferrioxamine;² patients who had a splenectomy were given prophylactic penicillin and instructed to report with symptoms of infection.

None of the group 1 patients had received regular transfusion, and their mean age at presentation (15.5 years) was higher than that of the other groups (table). The group 2 patients had received regular or intermittent transfusion (mean 48.6 units); after stopping transfusion there were no deleterious effects. The children in groups 1 and 2 showed normal development throughout the period of study. The adults showed normal sexual maturation; 11 of the women had a total of 17 successful pregnancies.

Group 3 comprised young patients who, either before or during the period of observation, showed increasing splenomegaly (mean 0.29 cm per month), anaemia (mean haemoglobin 49 g/L), or retarded development; splenectomy resulted in improvement of growth, QOL, and haemoglobin concentration ($p=0.001$; table). Group 4 patients also presented early and had received regular transfusion (mean 55.6 units); growth and development were unsatisfactory, on or off transfusion. Many of these patients had undergone splenectomy without apparent benefit. Reflecting iron loading (data to be reported elsewhere), two had established cirrhosis, two diabetes, and one hypothyroidism. Group 5 patients were not able to function adequately without blood transfusion. As a result of phenotypic instability, particularly among the younger patients, there were 13 changes of patient designation during the final 3 years of observation: seven children from groups 1, 2, 3, and 4 to group 5; four patients from group 2 to group 4 as the result of lack of sustained development, probably due to iron loading; one patient from group 2 (age 14 years) to group 3 following progressive splenomegaly; and one patient from group 4 (age 9.9 years) to group 3 after a successful splenectomy.

Major complications (table) included hypersplenism, iron loading, infection, malaria, leg ulcers, and gallstones. Of the four patients who died from infection (aged 11–52 years), three had undergone splenectomy; three (aged 10–42 years) died from iron loading, and one died from causes unrelated to thalassaemia.

To determine the reasons for the differences between the milder (groups 1 and 2) and more severe

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Panel: Protocol and descriptive classification of patients**Protocol**

1997

Clinical and genetic characterisation of 109 patients with haemoglobin E β thalassaemia
 Transfusions stopped because of iron-loading and uncertain basis for transfusions

1997–99

Regular assessment of QOL, growth, sexual development, and haematology

1999

Preliminary grading by severity

1999–2002

Regular assessment, as above

Reassignment of 13 patients to different groups

2003–04

Final analysis of original 109 patients and 31 patients who had presented during course of study

Clinical grading**Group 1**

Minimal transfusion: >20 years old, 0–20 units; <20 years old, 0–10 units

Adults: Sexually mature

Children: >3rd centile height, or growth rate >4 cm per year

QOL >5

Group 2

As for class 1, except transfusion history: >20 years old, >21 units; <20 years old, >11 units

Group 3

Post-splenectomy patients in whom the following changes occurred over 2 years: improvement in QOL >3 points; increase in growth >25%; improvement in growth rate to >3 cm per year

Group 4

Growth <3 centile for height; QOL >5; delayed sexual maturation

Group 5

QOL <5

Unable to function without transfusion

QOL=quality of life on a linear analogue scale, 0–10, validated by two observers within 7 days.³ The standard questions (in Sinhalese) included exercise tolerance, ability to perform day-to-day activities mood, appetite, etc. For babies the questions were modified to assess activity, feeding, comparison with peers, etc, and the point on the scale was determined by the mother.

phenotypes (groups 3–5), we made several comparisons (table). The mean steady-state concentration of haemoglobin, assessed by multiple estimations, in the milder groups was 63 g/L (SE 1.4, SD 11), and in the severe groups was 55 g/L (1.8, 12; $p=0.0001$). Data for patients born after 1990 were 66 g/L (SE 3.6, SD 10) and 51 g/L (3.8, 15), respectively ($p=0.01$). For those born before 1990, the values were 63 g/L (1.1, 8) and 57 g/L (1.9, 10), respectively ($p=0.01$). Although we identified several β thalassaemia mutations (table), they were all of the β^0 or severe β^+ variety and hence could not account for phenotypic variation. Much of the difference in haemoglobin values between the groups reflected the ability to produce haemoglobin F. We noted a correlation within the narrow range of haemoglobin values between the steady-state haemoglobin and the absolute level of haemoglobin F ($r=0.7$, $p=0.001$). The level of haemoglobin F showed a correlation with the

Xmn 1+/+ genotype at position –158 of the γ gene ($H\beta G2$) ($p=0.005$; figure); individuals with this genotype were restricted to groups 1 and 2, and their mean age was 33 years, compared with 19 years for the entire group. The frequency of the deletion forms of α^+ thalassaemia was too low for us to assess their contribution to phenotypic modification. However, restriction of α^+ thalassaemia alleles to groups 1, 2, and 3, and a lower gene frequency in the entire group (0.03), compared with those with β thalassaemia major (0.08)⁴ suggests that α thalassaemia is under-represented in this hospital population, raising the possibility of an ameliorating effect of α thalassaemia on haemoglobin E β thalassaemia. In ten patients studied, as in Thailand,⁶ we found no difference in the haplotypes of α globin stabilising protein between mild and severe phenotypes. The higher concentrations of bilirubin and incidence of gallstones in patients with the 7/7 genotype of the $UGT1A1$ promoter is reported elsewhere.⁵

Overall, 73 (57%) of the 128 patients studied showed positive serology for *P falciparum* and 101 (79%) for *P vivax* malaria. Of patients younger than 13 years, 28 of 36 studied had positive serology for *P vivax* and 16 of 39 had positive PCR for *P vivax* sequences during the period of observation; 13 of 39 had positive serology for *P falciparum*.

We noted a strong correlation between concentrations of erythropoietin and haemoglobin ($r=0.66$, $p=0.0001$) and a decline in erythropoietin response with age ($r=0.56$, $p=0.0001$); multi-regression analysis showed that age and anaemia were independent variables with respect to erythropoietin response. Further details of these findings, together with associated oxygen-affinity data, change in organ size with age, and cardiovascular status of older patients will be reported elsewhere.

Despite the wide diversity in age and previous medical intervention, and rapidly changing phenotypes in younger patients, some preliminary conclusions can be drawn. Most importantly, it is possible to stop transfusion in many patients with haemoglobin E β thalassaemia, with consequent saving of scarce resources. Surprisingly, the steady-state haemoglobin concentration, though significantly lower in the severe phenotypes than in the milder phenotypes, is of limited value in assessing severity. While it is clear that other genetic and environmental modifying factors remain to be discovered, age at presentation, γ gene promoter polymorphisms, α thalassaemia, and *UGTA1A* promoter polymorphisms are useful prognostic indicators. The possibility that malaria, particularly *P vivax*, can have a deleterious effect on the phenotype in early life requires further study; in the meantime careful attention should be paid to screening blood donors and malaria prophylaxis.

How should children with rapidly developing splenomegaly or growth failure be managed? While some respond to splenectomy, as in other populations,^{1,2} there is a high incidence of infection even after the institution of appropriate prophylaxis. Since transfusions can be stopped successfully, and since the preliminary adaptation data suggests a decline in the erythropoietin proliferative response with age, we suggest that a programme of transient transfusion through the period of maximum growth and erythroid expansion should be explored. Similarly, because of the small difference in haemoglobin concentrations between the mild and severe phenotypes, this form of thalassaemia intermedia might be particularly amenable to current experimental approaches to raise the level of haemoglobin F or total haemoglobin.²

Contributors

A Premawardhena, C A Fisher, A O'Donnell, and V Viprasakit did the laboratory studies. T E A Peto was responsible for the statistical analysis. S de Silva, M Arambepola, and W Perera were responsible for patient care throughout the study. L Merson and G Muraca were responsible for collating, recording, and preliminary analysis of the data, and the study was designed and supervised by A Premawardhena, S de Silva, N F Olivieri, and D J Weatherall.

	Group 1	Group 2	Group 3	Group 4	Group 5
Number	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 (8.5, 2.9)
Age at diagnosis (years)	1.5-48 (15.5, 13.4)	0.8-35 (9.9, 8.4)	0.4-5.6 (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 (6.0, 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, 3.8)	5-17 (11.4, 3.3)	4-15 (9.9, 3.3)
Facial deformity (0-4)*	0-2 (0.9, 0.7)	0-4 (1.5, 1.0)	0-4 (1.9, 1.2)	0-5-3.5 (1.9, 1.2)	1-3 (1.5, 0.9)
Splenectomised (%)	20	62	100	82	18
Time from splenectomy (years)	2-33 (13.3, 11.8)	2-19 (7.4, 3.3)	2-9 (3.1, 2.0)	1-13 (5.6, 3.4)	2-7 (4.0, 2.1)
Deaths (n)	2	3	0	2	1
History of major infection (n)	2	7	2	6	1
Leg ulcers (n)	8	20	3	3	0
Fractures (n)†	0	6	0	4	5
Gallstones (n)	5	15	1	3	0
Cholecystectomy (n)	3	1	0	1	0
Haemoglobin (g/L)	31-82 (65, 12)	36-83 (61, 10)	42-71 (58, 10)	44-80 (59, 12)	40-58 (47, 07)
Pre-splenectomy	35-43 (39, 06)
Post-splenectomy	65-76 (70, 15)	53-72 (64, 11)	64-87 (72, 12)	41-76 (61, 10)	40-55 (49, 13)
Haemoglobin F (g/L)	5-38 (20, 12)	2-37 (17, 7)	5-25 (14, 5)	4-27 (15, 3)	4-19 (9, 4)
Haemoglobin F (%)	8-60 (32, 13)	3-48 (29, 10)	10-34 (20, 6)	10-42 (22, 11)	6-42 (17, 8)
β thalassaemia mutation (n)					
IVS-1-5 (G→C)	13	27	10	13	8
IVS-1-1 (G→A)	10	10	3	5	2
Others	2	0	1	4	1
α + thalassaemia (n)	3	3	1	0	0
$\alpha\alpha/\alpha\alpha$ (n)	1	1	0	0	0
<i>Xmn</i> -1 +/+ (n)	6	5	0	0	0
<i>Xmn</i> -1 -/- (n)	2	2	2	5	4

Data are presented as range (mean, SD), or n=number of cases. *Facial deformity, including bossing of the skull, overgrowth of the zygoma and dental deformity was graded independently on a scale of 0-4 by two observers, and the mean value recorded. †Only one group 4 patient had fractures unrelated to severe trauma.

Table: Clinical, haematological, and genetic findings

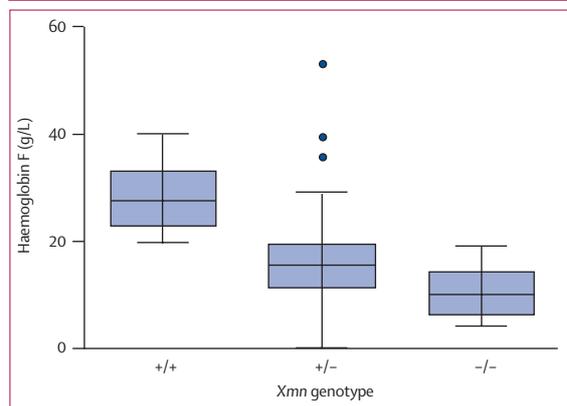


Figure: Relation between haemoglobin F and *Xmn*-1 polymorphism

This polymorphism reflects a C→T change at -158 in the region of the γ globin gene promoter, which is identified by the restriction enzyme *Xmn*-1.² The normal (C) arrangement is shown as - and the substitution of T as +. The boxes show the median and IQR. The outlying circles suggest that, as in other populations, there are factors, some genetic, that modify haemoglobin F values independent of the C→T polymorphism.²

Conflict of interest statement

We declare that we have no conflict of interest.

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