HB E β+ – THALASSAEMIA IN WEST MALAYSIA: CLINICAL FEATURES IN THE MOST COMMON BETA – THALASSAEMIA MUTATION OF THE MALAYS [IVS 1 - 5 (G → C)]

E George, H B Wong

ABSTRACT

Patients with the Hbβ+ [IVS 1-5 (G→ C)] clinically presented as beta-thalassaemia intermedia and remained asymptomatic in the absence of blood transfusions. With or without blood transfusions the patients were short and had moderate to marked thalassaemia facies. Children who received blood transfusions showed progressive iron loading with age. The serum ferritin and serum alanine transaminase levels were significantly raised in the patients who were given blood transfusions. In the presence of blood transfusions, and absence of adequate iron chelation therapy, splenectomy became an inevitable event at some stage of the disease because of increasing transfusing requirements.

Keywords: *Hb E beta thalassaemia, West Malaysia, asymptomatic.*

SINGAPORE MED J 1993, Vol 34: 500-503

INTRODUCTION

Haemoglobin (Hb) E beta – thalassaemia (thal) is a disease commonly observed among the population of far eastern countries such as Thailand, Vietnam, Cambodia, Laos, Malaysia, Indonesia, South China, Philippines and India. In the Malays the frequencies for beta – thal and Hb E are 3–9% and the occurrence of the compound heterozygote would be high in Malaysia[1]. Interaction of the beta E globin gene which behaves as a mild thal gene, with a gene for classical high Hb A2, beta – thal will result in a variety of clinical phenotypes which are determined by the type of beta–thal allele present, the haplotype of Hb E, coinheritance of alpha–thal and hereditary ovalocytosis. The most common beta thalassaemia mutation among the Malays is the mutation at position nucleotide 5 (G → C) of the first intervening sequence of the beta globin gene IVS 1-5 (G → C), which has a β+ phenotype[2].

Hb E – beta thalassaemia can be confusing for both patients and doctors, as the picture varies so much from one patient to the next. The lack of understanding of the underlying beta–thalassaemia mutations present in Hb E beta – thalassaemia has resulted in many patients with this disease being treated with regular blood transfusions similar to that practised for patients with homozygous – beta thalassaemia. Regular blood transfusions in the absence of intensive chelation therapy in patients with Hb E beta – thalassaemia ultimately result in death in the second decade secondary to haemosiderosis.

Department of Pathology
Faculty of Medicine
Universiti Kebangsaan Malaysia
50300 Kuala Lumpur
Malaysia

E George, FRCPA
Associate Professor

Department of Paediatrics
Faculty of Medicine
National University of Singapore
10 Kent Ridge Crescent
Singapore 0511

HB Wong, MBBS (Malaya), FRFPS (Glas), MRCP (Edin), FAMS, FRCP (Edin), FRACP (Aust), FRCP (Glas), FRCP (Lond), DCH (Lond)
Emeritus Professor

Correspondence to: Assoc Prof E George

This paper examines the clinical consequences of blood transfusion, effects of splenectomy and iron loading in patients with Hb E β+ [IVS 1-5 (G → C)].

MATERIALS AND METHODS

Patients

Eighteen Malay patients, with Hb E β+ [IVS 1-5 (G → C)] – thalassaemia from the Thalassaemia Clinic, Universiti Kebangsaan Malaysia, and the Paediatric Department, General Hospital, Kuala Lumpur were studied. The patients were divided into 2 groups (children/adults): Table I (Children aged 2 to 13 years, as seen at January 1989), Table II (the same group of children as studied in Table I, but as at January 1992); Table III (Adults aged 19 to 46 years, Group 1 were those who had no blood transfusion and Group 2 were adults with a history of some blood transfusions). A data profile chart was kept for each patient where information on liver profiles, serological assays, serum ferritin assays, heights and weights, and clinical studies were recorded. The height age for each patient was also determined: this was used to describe deficiency in height quantitatively by comparing patient's chronological age with his 'height age'. Example: A 13-year-old patient who is below the 3rd centile and has the same height as a 12-year-old growing on the 50th centile would have height age of 12 years. The height age hence would give both a rough measure of the growth deficiency, and a useful image of the patient.

Table I – Children with Hb Eβ [IVS 1-5 (G → C)] thalassaemia, 1989 (n = 8)

	Code	Age	Sex	Hb	SF	ALT	SB	S	Ht	Wt	HA	%	BT
1.	EB19NH	2	F	6.0	880	120	58	0	83	10	2	50	1
2.	EB25AM	3	F	6.6	520	22	31	0	88	11	2½	3-50	2
3.	EB18N2	3	F	5.6	640	93	43	0	89	12	2½	3-50	2
4.	EB13KNT	3	M	6.9	170	34	12	0	98	13	3	50-97	2
5.	EB23SI	4	F	7.1	1250	33	32	0	92	13	3	3-50	5
6.	EB10SA	12	M	6.1	6800	113	93	/11	133	30	9½	10-25	72*
7.	EB27MH	13	M	5.7	150	28	53	0	130	34	9½	< 3	0
8.	GMF	13	F	6.4	2400	180	61	/13	143	32	11	10-25	42*

M = male; F = female, Hb = gm/dl; SF = serum ferritin µg/L; ALT = serum alanine transaminase, U/L; SB = serum bilirubin, mmol/L; S = splenectomy. / Age = age when splenectomy was done; Ht = height, cm; Weight = kg; HA = height age; BT = blood transfusion, 1 unit = 350 ml; % = percentile; * = died 1991.

Table II – Children with Hb Eβ [IVS 1-5 (G → C)] thalassaemia, January 1992 (n = 8)

	Code	Age	Sex	Hb	SF	ALT	SB	S	Ht	Wt	HA	%	BT
1.	EB19NH	5	F		4785	180	67	0	99	14	4½	<3	14.4
2.	EB25AM	6	F		2500	49	25	0	96	16	4	<3	16.4
3.	EB18N2	6	F		4570	215	30	0	100	15	4½	<3	15.4
4.	EB13KNT	6	M		1230	128	38	0	110	17	6	<3	11.6
5.	EB23SI	7	F		3485	59	68	0	106	17	4½	<3	21.4
6.	EB10SA	14	M		5212	78	37	/11	138	31	10½	<3	90.3*
7.	EB2MH	16	M	6.1	400	8	47	0	136	27	10½	<3	0
8.	GMF	15	F		2000	126	86	/13	153	35	13½	10-25	52*

M = male; F = female, Hb = gm/dl; SF = serum ferritin µg/L; ALT = serum alanine transaminase, U/L; SB = serum bilirubin, mmol/L; S = splenectomy. / Age = age when splenectomy was done; Ht = height, cm; Weight = kg; HA = height age; BT = blood transfusion, 1 unit = 350 ml; % = percentile; * = died 1991.

Table III – Adults with Hb Eβ [IVS 1-5 (G → C)] thalassaemia

	Code	Age	Sex	Hb	SF	ALT	SB	S	Ht	Wt	HA	%	Others
Group 1		**Absence of blood transfusions (n = 6)**											
1.	EB22N	19	M	8.3	110	20	66	0	163	54	18	10	α - thal
2.	EB R	23	F	7.3	150	13	38	0	152	49	13	10-25	G3P3
3.	EB K	25	M	8.5	210	14	54	0	161	47	14	3-10	normal sexual characteristics
4.	EB42H	27	F	7.9	180	12	99	0	150	49	12½	10-25	G2P2
5.	N2	29	F	7.0	120	19	30	0	151	48	12½	10-25	G1P1
6.	EB30H	32	F	9.4	380	38	59	0	153	62	13	10-25	G3P3
Group 2		**With blood transfusions (n = 4)**											
7.	2Y	22	F	7.6	4100	157	48	/11	153	43	12½	<3	
8.	MI	36	F	5.4	1900	17	45	/35	157	40	12½	<3	
9.	SY	42	M	6.1	2000	64	34	/32	156	42	12½	<3	
10.	AG	46	M	6.2	3915	64	61	/36	162	43	14	3-10	

M = male, F = female, Hb = gm/dl; SF = serum ferritin, µg/L; ALT = serum alanine transaminase = U/L; S = Splenectomy, O = splenectomy not done; /Age = age when splenectomy was done; Ht = cm; Wt = weight, kg; HA = height age; SB = serum bilirubin, mmol/L;

Haematological

Red cell indices were collected on Coulter counters M530 in 1989, and on JR after 1990. Electrophoresis of the haemoglobins was carried out on cellulose acetate strips pH 8.6 and by isoelectric focusing (IEF) pH 6-8[2-4]. Quantitation of HbA and other globin chains were by reverse phase high performance liquid chromatography (HPLC)[5,6]. The level of HbF was estimated by alkali denaturation procedure[3]. The quantitative determination of serum ferritin was by microparticle enzyme immunoassay (MEIA) (Imx system ferritin, Abbot Laboratories, Diagnostics Division, Abbott Park, IL 60064, USA) done in duplicate for each sample.

Statistical Analysis

To compare the features between the patients who had blood transfusion and those without blood transfusion, non parametric analysis used the Wilcoxon's rank – sum test. The Spearman's rank correlation coefficient was used to evaluate the relationship between the serum ferritin levels to age and to the units of blood transfused. All data were expressed as means and SDs (Table IV). Statistical significance was assessed at $p < 0.05$.

RESULTS

Haematological, biochemical and clinical data are shown on Tables I, II, III and IV. A classical thalassaemia picture was seen in the peripheral blood films: the red cells showed moderate to marked anisopoikilocytosis, hypochromia, polychromasia, and microcytosis. There were target cells and some mis-shaped red blood cells. In the patients with Hb E β+ [IVS 1-5 (G → C)] – thalassaemia minimal amounts between 3.5 – 5.5% of HbA were synthesised. The range of HbF seen was 4.5 – 75%, the values in the lower range being seen in the patients with alpha thalassaemia associated with Hb E β+ [IVS 1-5 (G → C)]. In a steady state, most patients had haemoglobin levels above 7 gm/dl. The majority of patients were short, with moderate thalassaemic facies and hepatosplenomegaly. Minimal clinical findings were seen in the patient who had both an IVS 1-5 (G → C) mutation and alpha thalassaemia (Table III, EB22 N). Seven (39%) who had no blood transfusion, had serum ferritin levels that were below 500 ug/L. Children who had received blood transfusions in the absence of iron chelation therapy showed a positive correlation of age to serum ferritin levels (1989, $r_s = 0.8$; 1992, $r_s = 0.3$). Adult patients who had received blood transfusions had significantly higher serum ferritin levels when compared to those patients without a history of blood transfusions ($p < 0.01$). In the children there was a positive correlation of the serum ferritin levels to the units of blood transfused (1989, $\alpha = 0.01$, $r_s = 0.06$; 1992, $\alpha = 0.01$, $r_s = 0.14$). In the absence of blood transfusions all adults had serum alanine transminase levels that were < 40 U/L but 3 (75%) of adults who received blood transfusions had serum alanine transaminase levels > 40 U/L. Splenectomy was seen in 7 (39%) of patients, and there were two deaths from overwhelming infection among the splenectomised patients. The viral hepatitis markers tested for are shown in Table V. The mothers of those who were tested positive for HBsAg showed no HBV markers.

Table IV– Serum ferritin and serum alanine transaminase levels in patients with Hb E β [IVS 1-5 (G → C)] thalassaemia

Mean ± 1SD	1989	1992
a) Children (GD)	**n = 8**	**n = 8**
SF µg/L	1601.3±2221.8	3022.7±1769.5
ALT u/L	77.8± 57.5	105.3± 69.7
b) Children (NBT, NC)	**n = 1**	**n = 1**
SF µg/L	150	400
ALT u/L	28	8
c) Children (BT±C)	**n = 7**	**n = 7**
SF µg/L	1808.5±2314.8	3397.4±1530.6
ALT u/L	85 ± 58.2	119.3± 62.2
d) Adults (n=10)	**NBT (n=6)**	**BT (n=4)**
SF µg/L	191.6±99.5	2978.8±190.9
ALT u/L	19.3± 9.7	75.5± 58.6

SF = serum ferritin; ALT = serum alanine transaminase; BT = blood transfusions; NBT = no blood transfusion; NC = no iron chelation; C = iron chelation.

Table V – HBV and HBC markers in patients with Hb E β+ [IVS 1-5 (G → C)] – thalassaemia n = 8

BT	HBs Ag	Anti HBs	Anti HBc	Anti HBC
none	0	2(11)	0	0
with	3(16.6)	2(11)	2(11)	1(5.5)

BT = blood transfusion; percentage of markers within parenthesis.

DISCUSSION

Haemoglobin E – beta thalassaemia shows a remarkable degree of variability of clinical expression. At its worst, it is similar to homozygous beta – thalassaemia. Affected children suffer from severe anaemia from an early age and have marked skeletal deformities with typical thalassaemic facies[7,8]. Detailed early descriptions of patients with haemoglobin E – beta thalassaemia were from Thailand, with some clinical descriptions of the disease from other racial groups[9-11]. All these reports emphasise remarkable variability in the clinical expression of the disease, which is now explainable on the basis of the mutation of beta thalassaemia present in these ethnic groups. The association of haemoglobin E with β° – thalassaemia should theoretically produce more severe clinical manifestations than β+ thalassaemia. In China and Thailand the predominant beta – thalassaemia mutation is β°[12,13]. In contrast our studies in West Malaysia showed that the beta – thalassaemia mutation, IVS 1-5 (G → C) which has a β+ phenotype is the most common beta thalassaemia mutation in the Malays[2]. The beta – thalassaemia mutation IVS 1-5 (G → C) is also the predominant mutation seen in Indonesia[14] and in Melanesia[15], a feature in keeping with the common population migration and the natural selection of this mutation by malaria in this region. In this mutation HbA is synthesised in small amounts from 2.7–5.8%. The beta E gene (β^{E}) acts as a mild β+ determinant, with the globin chain synthesis slightly imbalanced in subjects who are haemoglobin E heterozygotes (alpha/non–alpha ratio 1.03 – 2.19)[16]. The clinical manifestations of haemoglobin E β+ thalassaemia in a steady state is likely to be thalassaemia intermedia and not transfusion dependent. In addition, these populations with haemoglobin E – beta thalassaemia also have alpha thalassemia. In Thailand[13] the incidence for alpha thalassaemia is 20 – 30% (3.5% for α°, and 16% for α^{+}), and in West Malaysia it is 20 – 27% (2% for α°, and 18% for α^{+}). Concomitant coinheritance of alpha thalassaemia can ameliorate the severity of haemoglobin E – beta thalassaemia. The coinheritance of the α° – thal gene leads to a greater alleviation of the severity than that by the α^{+} – thal gene. Haemoglobin E – beta thalassaemia patients who carry the α° – thal gene are not likely to be found in a hospital-based population. In this study none of the patients were associated with the α° – thal gene, and the α^{+} – thal gene was seen only in one patient (Table III, EB N), a young Malay adult, diagnosed from investigations following an episode of febrile illness. He had no thalassaemic facies, hepatosplenomegaly, and no history of blood transfusions.

In this study, significant iron overload was not a feature in the absence of blood transfusions and patients had serum ferritin levels less than 500 ug/L. Sexual maturation was normal: four adult females with no history of blood transfusions, showed normal sexual characteristics, and had produced children (Table III).

Growth appeared to be normal until 5 years of age with and without blood transfusions. Growth deficiency became manifest after the age of 9. In the presence of blood transfusions and lack of iron chelation therapy or inadequate chelation therapy, 50% of the patients by the age of 13 were below the 3rd centile (Tables I and II). Adults, however, who had not been transfused had moderate thalassaemic facies, intact spleens which were enlarged and a growth height age corresponding to 12 – 14 years (Table III). It is possible for patients who are on regular iron chelation therapy with Desferal, to look 'cosmetically normal' with regular blood transfusions which keep haemoglobins above 8 gm/dl. On this regime patients will have normal growth and development and show minimal enlargement of liver and spleen. However the costs of iron chelation therapy are exorbitant in Malaysia and this form of therapy if practised will only be available to a minority of patients. In this study only one patient was on iron chelation therapy with Desferal. The majority of patients with regular blood transfusions show evidence of iron overload, and evidence of transfusional related viral hepatitis[17]. Studies in Malaysia have shown a moderate prevalence of Hepatitis B virus infection ranging from 3 to 11% depending on the ethnic group in Peninsular Malaysia[18]. To ensure safe blood transfusion, screening for HBsAg was introduced in the National Blood Services Centre in Kuala Lumpur from 1972. Inspite of the careful HBsAg screening which now is carried out on all blood donors, in our study 16.6% of the patients with Hb E – beta thalassaemia were HBsAg positive and 18% have been exposed to HBV infection. In some cases the HBsAg positive donors may not be identified on screening. On the other hand, the patients might have got their HBV infection by other routes than through blood transfusion. Transmission from mother to neonate during birth is one of the most efficient mode for HBV transmission. In this study none of the mothers were positive for HBsAg. However the HBV status at the time of delivery was not available. Seven (87.5) of the children at 1992 and 3 (75%) of the adults who were on blood transfusions showed raised serum alanine transaminase levels (Tables II and III) and liver biopsies done on two patients who had persistently raised alanine transaminases showed secondary haemachromatosis in both, chronic active hepatitis in one (Table II, GMF, who was also positive for anti HBC), and micronodular cirrhosis in the other (Table II, EB 10 SA). Screening for HBV markers is vital at the start of blood transfusion treatment and hepatitis B vaccination should be carried out in susceptible patients. With the current lack of availability of Desferal to most patients with Hb E beta – thalassaemia, it is prudent not to transfuse blood as this study has shown that the majority of Hb E β+ [IVS 1-5 (G → C)] - thalassaemia patients will most likely be asymptomatic with blood transfusions. Those who are symptomatic and show a need for regular blood transfusions, could be managed on a low to moderate transfusion practice (pretransfusion Hb > 6 gm/dl, and post-transfusion Hb 8 – 10 gm/dl). However there is a need for adequate clinical trials locally to determine the ideal blood transfusion practice, especially so now with the current information of the spectrum for the beta thalassaemia mutations present in Malaysia. Splenectomy appeared as a regular feature at some stage of the disease in patients who were on blood transfusions. Following splenectomy, the haemoglobin levels remain between 6 – 7 gm/dl. Both patients who had died in this study were splenectomised and succumbed to overwhelming infection.

The serum ferritin levels in patients with Hb E – beta thalassaemia who were not receiving regular blood transfusion were less than 500 ug/L. However, it is important for long term follow-up of adult haemoglobin E beta – thalassaemia patients with regular monitoring of serum ferritin levels despite the absence of blood transfusion therapy as it has been reported that patients with thalassaemia intermedia may show evidence of iron overload with increasing age through the absorption of iron from the gut[19].

In summary, patients with Hb E β [IVS 1-5 (G → C)] – thalassaemia were asymptomatic in the absence of blood transfusions. Regular blood transfusions in the absence of adequate chelation therapy with Desferal resulted in the majority of patients requiring splenectomy at some stage of the disease and a possibility of death in the second decade as a consequence of organ failure secondary to iron overload or to overwhelming infection. The prognosis of patients with Hb E β+ thalassaemia has improved with advances in management as a result of the increased understanding of the beta – thalassaemia mutations present in West Malaysia. In this study the main aspects of management were no blood transfusions or maintenance blood

transfusions, iron chelation therapy to combat transfusional siderosis, and splenectomy. The dominating problem was the control of transfusional iron overload secondary to blood transfusions.

We conclude, as patients with Hb E β+ [IVS 1-5 (G → C)]–thalassaemia are usually asymptomatic with low haemoglobins, it may not be necessary to put them on maintenance transfusion because of the dangers of iron overload and the absence of adequate iron chelation therapy programme in the country.

ACKNOWLEDGEMENTS

This research was supported in part by the grants U.K.M. 55/85, I.R.P.A. 3–07–03–024, and I.R.P.A. 3–07–03–072. The serum ferritin study is a contribution from the M.D. programme, National University of Singapore of Dr. E. George.

REFERENCES

1. George E, Khuziah R. Malays with thalassaemia in West Malaysia. J Trop Geogr Med 1984; 26: 123-5.
2. Yang KG, Kutlar F, George E, Wilson JB, Kutlar A, Stoning TA, et al. Molecular characteristics of β - globin gene mutations in Malay patients with Hb E β – thalassaemia and thalassaemia major. Br J Haematol 1989; 72: 73-80.
3. Dacie JV, Lewis SM. Practical Haematology. London : Churchill and Livingstone, 1975.
4. Basset P, Beuzard Y, Garel MC, Rosa J. Isoelectric focusing of human haemoglobin: its application to screening, to the characterisation of 70 variants, and its study of modified fractions of normal haemoglobin. Blood 1978; 51: 971-82.
5. Shelton JB, Shelton JR, Schroeder WA. High performance liquid chromatographic separation of globin chains on a large pore C 4 column . J Liquid Chromatography 1984; 7 : 169-77.
6. Kutlar F, Kutlar A, Huisman THJ. Separation of normal and abnormal chains by reverse high performance liquid chromatography. J Liquid Chromatography 1986: 147-53.
7. Weatherall DJ, Clegg JB. The thalassaemia syndrome. 3rd Ed. London: Blackwell Scientific Publications, 1981.
8. Minnich V, Na-Nakorn S, Chongchareonsuk S, Kochaswni S. Mediterranean anaemia: a study of 32 cases in Thailand. Blood 1954; 9: 1-3.
9. Lehman H, Singh El. Hb E in Malaya. Nature 1956; 178: 695- 7.
10. Lie - Injo LE. Pathological haemoglobins in Indonesia. Nature 1959; 13; 1125-6.
11. Chatterjea JB. Haemoglobinopathies, Glucose - 6 - phosphate dehydrogenase deficiency and allied problems in the Indian sub-continent. Bull World Health Organisation 1966; 35: 837-9.
12. Kazazian HH Jr. Molecular basis and prenatal diagnosis in 1990. Semin Haematol 1990; 27: 209-28.
13. Fucharoen S, Winichagoon P, Thonglairuam V. β - thalassaemia associated with α - thalassaemia in Thailand. Haemoglobin 1988; 12 (5&6) : 581-92.
14. Lie - Injo LE, Cai SP, Kan YW. β - thalassaemia mutations in Indonesia and their linkage to β haplotypes. Am J Human Genet 1989; 45: 971-5.
15. Hill AVS, Bowden DK, O'Shaughnessy DF, Weatherall DJ, Clegg JB. β - thalassemia in Melanesia: associated with malaria and characterisation of a common variant IVS 1- 5 (G → C). Blood 1988; 72: 9-14.
16. Traiger J, Wood WG, Clegg JB, Weatherall DJ, Wasi P. Defective synthesis of Hb E is due to reduced levels of mRNA. Nature 1980; 288: 497-9.
17. George E, Ilina I, Yasmin AM, George R, Duraisamy G. Hepatitis B infection in multitransfused thalassaemics. Med J Malaysia 1988; 43: 284-7.
18. Lopez CG. Epidemiology of persistent hepatitis B virus infection. Malaysia J Pathol 1985; 7: 7-9.
19. Pippard MJ, Callender ST, Warner GT, Weatherall DJ. Iron absorption and loading in β - thalassaemia intermedia. Lancet 1979; 20; 819-21.