CLINICAL AND HEMATOLOGICAL PHENOTYPE OF HOMOZYGOUS HEMOGLOBIN E: REVISIT OF A BENIGN CONDITION WITH HIDDEN REPRODUCTIVE RISK

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Abstract. Hemoglobin E (HbE) is one of the most prevalent β-globin variant, which is widely distributed in Southeast Asia especially in Thailand. Homozygosity for this variant is common and may occur with iron deficiency. In order to study clinical and hematological phenotypes without the confounding effect of iron deficiency, investigations were carried out before and after iron supplementation for 2 months. The effect of G6PD deficiency and coinheritance of α-thalassemia in homozygous HbE were also studied. HbE homozygotes were clinically benign, never had been transfused and had no hepatosplenomegaly. Out of 76 HbE homozygotes, hematological parameters of 7 individuals with iron deficiency improved after iron supplementation. Hemoglobin analysis revealed that HbE was the main hemoglobin detected, but 12 subjects were found to have a substantial percentage of HbF, which might lead to misdiagnosis as HbE/β-thalassemia. Both clinical and hematological phenotypes of simple homozygous HbE did not differ from those who also inherited α-thalassemia and/or G6PD deficiency. It is necessary to perform a comprehensive DNA analysis for α-thalassemia in cases of homozygous HbE when their partner is suspected of having α-thalassemia 1 gene.

INTRODUCTION

Thalassemia and hemoglobinopathy are the most common single gene disorder affecting millions of people. The World Health Organization has recently estimated that nearly 7% of the world's population carry either one or another of these abnormal genes (WHO, 1989). Two major forms of thalassemia, α- and β-thalassemia, characterized by reduction or complete abolishment of expression of globin genes result from more than 250 different mutations, predominantly missense, nonsense and deletion of their structural genes (Weatherall, 2000).

In the case of β-thalassemia, homozygosity and compound heterozygosity of β^{o}-thalassemia and variant hemoglobin (Hb), *viz*. HbE ($\beta^{26\ Glu \rightarrow Lys}$), HbS ($\beta^{6\ Glu \rightarrow Val}$), or HbC ($\beta^{6\ Glu \rightarrow Lys}$), produces a major health burden in many countries as affected individuals can present with an extensive variety of clinical syndromes, ranging from minor chronic hemolytic anemia, moderate anemia with occasional transfusion, to very severe anemia with a life-long transfusion requirement (Weatherall, 2000). In Southeast Asia, HbE

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is extremely common with an average allele frequency of 5-10% and reaching 50% in some areas as this allele is believed to provide protection against malaria, which has been and remains prevalent in this region (Fucharoen *et al*, 1988). In addition, several different alleles causing α-thalassemia are also common in the same region owing to a similar selection by malaria in the past (Clegg and Weatherall, 1999). Therefore it is not uncommon to encounter a coinheritance of both α- and β-thalassemia in the same individual and this provides an added difficulty in providing proper genetic counseling and appropriate prenatal control of severe thalassemia, especially in Thailand (Winichagoon *et al*, 2000).

In the present study, we analyzed the clinical phenotypes and hematological parameters of 76 Thai individuals with homozygous HbE. In addition we carried out further analyses to determine the effects of iron status, glucose-6-phosphate dehydrogenase (G6PD) deficiency and coinheritance of α-thalassemia on their hematological picture and clinical presentation.

MATERIALS AND METHODS

Subjects

As part of an ongoing project on the natural history and preventive control program of HbE/β-thalassemia in Thailand, we diagnosed 76 individuals as having homozygous HbE. They were either sibling or parents of patients with HbE/β-thalassemia or were detected during routine screening. This study was approved by a local ethics committee at Siriraj Hospital, Thailand.

Hematological analysis

Complete hematological studies were performed on peripheral blood samples collected using EDTA as anticoagulant. Red blood cell indices were analyzed using an automated blood cell counter (Sysmex F280, Japan). Hb analyses were performed using starch gel-electrophoresis in Tris-borate-EDTA buffer, pH 8.6 followed by staining with orthodianisidine (Smithies, 1955). As the electrophoretic migration of HbE is similar to that of HbA_2, in order to determine the amounts of HbE and HbF, these Hb species were quantified by elution following cellulose acetate electrophoresis (ICSH, 1978). In addition, the levels of HbF were determined by alkaline denaturation method (Betke *et al*, 1969).

Serum ferritin was determined during a steady state (no current infection, no history of inflammatory diseases or liver dysfunction) by ELISA technique (Roche Diagnostic, Mannheim, Germany). Intra- and inter-assay quality controls were performed using standard sera containing 0, 7.8, 16.8, 272 and 966 μg/dl of ferritin). Individuals with iron deficiency, defined on the basis of serum ferritin of less than 16 μg/dl (Hallberg *et al*, 1993) were given an iron supplement (2 mg of ferrous sulfate/kg/day) for 60 days before being reevaluated for their hematological phenotype.

Analysis of HbE mutation

DNA diagnosis of HbE was achieved by polymerase chain reaction (PCR) amplification of β^E-globin gene followed by digestion of the amplicon with restriction enzyme *Mnl* I since the G→A substitution abolishes this enzyme cleavage site (Winichagoon *et al*, 1989). PCR was performed using forward primer HbE-F (5'-CATTTGCTTCTGACACA ACTG-3') and reverse primer HbE-R (5'-TTGAGGTTGTCCAGGTGAG-3') in a reaction volume of 25 μl containing 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl (pH 8.3), 200 μM of each dNTP, 25 pmol of each forward and reverse primer and 2.5 U of *Taq* polymerase (Roche Diagnostic, Mannheim, Germany) and 100 ng of genomic DNA.

Thermocycling (carried out in Dyad Diciple™ Cycler, MJ Research, Boston, USA) consisted of an initial denaturation step of 95ºC for 5 minutes, followed by 30 cycles of 95ºC for 60 seconds, 50ºC for 60 seconds and 72ºC for 60 seconds, with a final step of 72ºC for 10 minutes. Amplicon (427 bp) was subsequently digested with 1.25 U of *Mnl* I at 37ºC for at least 4 hours. Digested products were electrophoresed in 2% agarose gel, stained with 1% ethidium bromide and visualized by Syngene gel documentation and analysis system (Synoptic group, Cambridge, UK).

Analysis of α-thalassemia genes

To determine co-inheritance of common α-thalassemia in homozygous hemoglobin E subjects, 5 common deletional α^0-thalassemia including $--^{SEA}$, $--^{FIL}$, $--^{THAI}$, $--^{MED}$ and $--^{(20.5)}$ and two α^+-thalassemia ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) were screened using multiplex GAP-PCR analysis as previously described (Liu *et al*, 2000). In addition, two sets of mismatched PCR-restriction fragment length polymorphism (RFLP) were employed to detect two common non-deletional α-thalassemia ($\alpha^T\alpha$), HbConstant Spring and Hb Pakse, due to termination codon mutation by substitution of TAA to CAA and TAT respectively (Viprakasit *et al*, 2002). All analyses were conducted in parallel with known positive controls of both mutations previously confirmed by direct DNA sequencing of the mutant α-globin genes.

RESULTS

Clinical and hematological phenotypes in HbE homozygotes

Complete physical examination was performed on 76 homozygous HbE individuals (35 females and 41 males, age ranging 1- 43 years). All had uneventful medical histories and never receive blood transfusion. No subject was found to have hepatosplenomegaly and did not have a history of deteriorating anemic symptom during feverish episode or infection. Diagnosis of homozygous HbE was confirmed in every case by PCR. The amplicon (427 bp) contains two *Mnl* I restriction sites, one at the 5'-end used as an internal control for digestion (135 bp) and the other linked to codon 26 (171 and 62 bp). HbE mutation abolishes this latter *Mnl* I site and thus digestion produced 233 bp and 135 bp fragments (Fig 1).

Iron deficiency in HbE homozygotes

In order to study the 'real' hematological phenotype resulting from homozygous HbE, we excluded the influence of iron deficiency, by determining serum ferritin level at steady state of all subjects.

On the first visit, 7 individuals were diagnosed as having iron deficiency anemia (serum ferritin < 16 μg/l). This group was composed of 2 women who had a history of hypermenorrhea and 5 children, ages 1-3 years. The cause of iron deficiency in these children was not determined further; although a combination of nutritional problem with possible parasitic infestation was

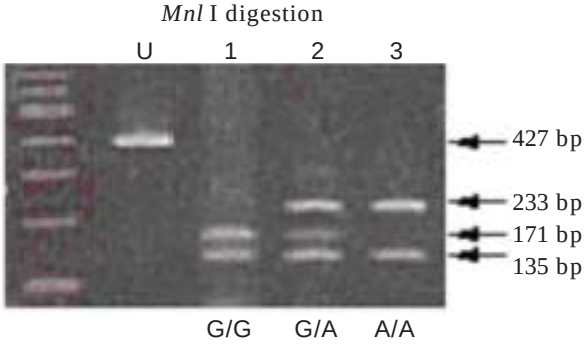

Fig 1–PCR diagnosis of hemoglobin E. 2.5% Agarose gel shows the fragments that result from *Mnl* I digestion of the region of codon 26 amplification: Lane U, undigested amplicon; lane 1, sample without HbE; lane 2, heterozygous HbE; lane 3, homozygous HbE.

Table 1
Comparison of hematological parameters before and after iron supplement in HbE homozygotes with iron deficiency anemia.

Hematological parameter	Iron supplementation		*p*-value
	Before	After	
Hb (g/dl)	9.0 ± 2.3	11.4 ± 0.6	0.027
PCV (%)	28.8 ± 5.5	34.4 ± 1.6	0.030
RBC ($x10^{12}/mm^3$)	5.7 ± 0.8	6.1 ± 0.7	0.085
MCV (fl)	51.0 ± 9.8	56.4 ± 6.1	0.030
MCH (pg)	16.0 ± 4.1	18.7 ± 2.3	0.026
MCHC (g/dl)	30.9 ± 2.6	33.2 ± 1.0	0.037
RDW (%)	19.2 ± 1.8	18.6 ± 1.6	0.190
Serum ferritin (μg/l)	6.5 ± 3.6	38.9 ± 29.4	0.022

Data are presented as mean ± SD (n = 7). P-value < 0.05 is considered statistically significant. (Hb, hemoglobin; PCV, packed cell volume; RBC, red blood cell count; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red cell distribution width).

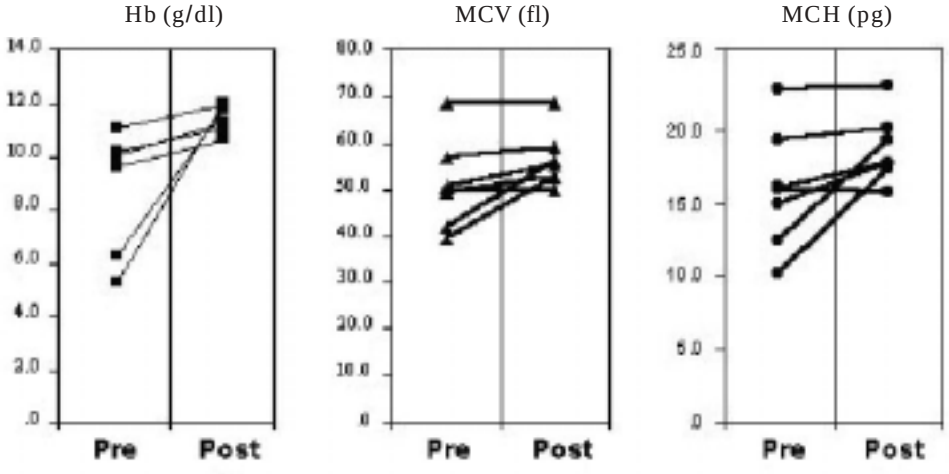

Fig 2–Comparison of hematological parameters before and after iron supplement in seven HbE homozygotes with iron deficiency anemia. The Hb level, mean cell volume (MCV) and mean cell hemoglobin (MCH) improved after two months of iron supplement.

suspected. This was supported by the observation that all children responded well with iron supplementation and antiparasitic therapy. Hematological parameters, *viz* Hb level, mean cell volume (MCV) and mean cell hemoglobin (MCH), of the 7 subjects improved substantially after two months of iron supplement (Fig 2). Serum ferritin level and most of the hematological parameters, except red cell distribution width (RDW) and red blood cell count (RBC), were significantly increased after iron supplement (Table 1).

Table 2
Comparison of hematological parameters before and after iron supplement in HbE homozygotes without iron deficiency anemia.

Hematological parameter	Iron supplementation		*p*-value
	Before	After	
Hb (g/dl)	11.3 ± 1.3	11.4 ± 1.3	0.708
PCV (%)	34.6 ± 3.9	34.3 ± 4.0	0.823
RBC ($x10^{12}/mm^3$)	5.98 ± 0.58	5.88 ± 0.64	0.057
MCV (fl)	57.8 ± 4.7	58.5 ± 4.9	0.124
MCH (pg)	18.9 ± 1.7	19.4 ± 1.7	0.000
MCHC (g/dl)	32.6 ± 1.2	33.2 ± 1.2	0.001
RDW (%)	18.0 ± 1.2	17.9 ± 1.4	0.392
Serum ferritin (μg/l)	116 ± 124	157 ± 140	0.000

Data are presented as mean ± SD (n = 69). P-value < 0.05 is considered statistically significant. Abbreviations are as defined in Table 1.

Table 3
Comparison of hematological parameters before and after iron supplement in all HbE homozygotes.

Hematological parameter	Iron supplementation		*p*-value
	Before	After	
Hb (g/dl)	11.0 ± 1.5	11.4 ± 1.3	0.124
PCV (%)	34.0 ± 4.3	34.3 ± 3.8	0.823
RBC ($x10^{12}/mm^3$)	5.98 ± 0.60	5.93 ± 0.67	0.176
MCV (fl)	56.9 ± 5.8	58.0 ± 5.2	0.019
MCH (pg)	18.5 ± 2.2	19.3 ± 1.9	0.000
MCHC (g/dl)	32.5 ± 1.4	33.2 ± 1.2	0.000
RDW (%)	18.1 ± 1.3	18.0 ± 1.4	0.403
Serum ferritin (μg/l)	104 ± 120	151 ± 139	0.000

Data are presented as mean ± SD (n = 76). P-value < 0.05 is considered statistically significant. Abbreviations are as defined in Table 1.

In order to study real hematological parameters of HbE homozygote, all subjects were treated with iron for 2 months even though serum ferritin levels were not less than 16 μg/l. MCH, MCHC and serum ferritin level for subjects without iron deficiency anemia were significantly increased after iron supplement (Table 2). Hematological data for all homozygous HbE subjects showed significant increase in MCV, MCH, MCHC and serum ferritin level after iron supplement (Table 3).

G6PD deficiency in male individuals with homozygous HbE

Among 41 males with homozygous HbE, there were 10 (24%) with G6PD deficiency, having G6PD ranging from 0 to 9.4

Table 4
Comparison of hematological parameters after iron supplementation in HbE homozygotes with different α-globin genotypes.

α-Globin genotype	*n*	Male (%)	Hb (g/dl)		MCV (fl)		MCH (pg)		MCHC (g/l)		RBC ($x10^6$)		RDW (%)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A. αα/αα	54	29 (54)	11.2	1.2	58.0	4.9	19.3	1.7	33.2	1.2	5.86	0.67	18.1	1.5
B. -α/αα	18	9 (50)	11.7	1.3	59.0	5.5	19.5	1.9	33.1	0.7	6.03	0.62	17.4	1.0
p-value (A and B)			0.152		0.398		0.598		0.691		0.191		0.091	
C. -α/-α or --/αα	4	3 (75)	11.7	1.7	59.0	6.1	20.1	2.9	33.8	1.9	5.84	0.52	18.8	1.5
p-value (A and C)			0.623		0.736		0.490		0.289		0.782		0.203	

P-value < 0.05 is considered statistically significant. Abbreviations are as defined in Table 1.

IU/dl (mean ± SD = 1.7 ± 3.7 IU/dl), compared with activities of 153.1-324.0 IU/dl (mean ± SD = 207.3 ± 49.5 IU/dl) in the other 31 individuals. Comparing homozygous HbE male individuals between groups with normal G6PD and G6PD deficiency (with identical α-globin genotypes, see below), no additional effect of G6PD deficiency upon hematological parameters was observed (data not shown).

Coinheritance of α-thalassemia in HbE homozygotes

Using multiplex GAP-PCR analysis for deletional α-thalassemia and mismatch-PCR-RFLP for detection of HbCS and Pakse, 22 (29%) individuals who also coinherited α-thalassemia were identified. Abnormal α-globin genotypes included heterozygous α^+ thal ($-\alpha^{3.7}/\alpha\alpha$, *n* = 16), heterozygous HbCS ($\alpha^{CS}\alpha$/aa, *n* = 2), homozygous α^+-thal ($-\alpha^{3.7}/-\alpha^{3.7}$, *n* = 1), compound heterozygous α^+-thal and HbCS ($-\alpha^{3.7}/\alpha^{CS}\alpha$, *n* = 2) and heterozygous α^0-thal ($--^{SEA}/\alpha\alpha$, *n* = 1). Hb Pakse and other deletional α-thalassemias (THAI and FIL deletion) were not found in the subjects studied. In order to compare hematological parameters in HbE homozygotes with and without α-thalassemia, we categorized interacting α-thal determinants into two groups, depending on the numbers of affected α-globin genes: single affected α-globin gene ($-\alpha^{3.7}/\alpha\alpha$ and $\alpha^{CS}\alpha/\alpha\alpha$ *n* = 18) and two affected α-globin genes ($-\alpha^{3.7}/-\alpha^{3.7}$, $\alpha^{3.7}/\alpha^{CS}\alpha$ and $--^{SEA}/\alpha\alpha$, *n* = 4). There is no statistically significant difference between the two groups of HbE homozygotes in Hb level or other red blood cell indices (Table 4).

HbF level in HbE homozygotes

There were 12 subjects (2 adult females and 10 children), ages 1-27 years, with high HbF level (8.3-24.6%) (Table 5). Among these children, there were 7 children in the age range of 1-2 years. Six subjects were re-evaluated for HbF level after the age of 3 years

Table 5
Hematological parameters of HbE homozygotes with high percent HbF.

Cases	Age (yrs)	Sex	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/l)	HbE (%)	HbF (%)	Ferritin (μg/l)	α-Globin genotypes	*Xmn I*
1. P.P	1	M	9.1	54.7	17.0	34.0	91.7	8.3	54	$-\alpha^{3.7}/\alpha\alpha$	+/-
2. P.B.	1	M	11.1	50.0	15.8	31.6	89.1	10.9	64	αα/αα	+/+
	5		11.2	52.8	18.2	34.5	100				
3. J.P.	1	F	11.6	53.0	17.4	32.0	86.1	13.9	21	αα/αα	+/+
	6		10.9	57.9	18.6	32.2	97.8	2.2			
4. W.N.	1	M	12.0	58.6	18.1	32.8	75.4	24.6	29	αα/αα	+/+
	6		10.2	57.5	17.7	30.8	93.3	6.7			
5. J.P.	2	M	10.8	54.0	18.5	34.2	90.7	9.3	61	αα/αα	+/-
6. Y.Y.	2	M	12.0	59.1	20.1	34.1	89.7	10.3	38	$-\alpha^{3.7}/\alpha\alpha$	+/-
	7		12.3	76.9	21.4	31.5	98.7	1.3			
7. S.A.	2	M	10.0	59.4	19.9	33.4	81.0	19	123	αα/αα	+/-
	5		10.9	59.0	18.2	30.8	98.3	1.7			
8. C.L.	3	M	11.0	54.4	19.3	35.5	87.4	12.6	47	αα/αα	+/+
	7		9.9	58.4	18.6	31.9	98.4	1.6			
9. T.M.	3	M	10.7	56.6	19.5	34.5	82.4	17.6	83	αα/αα	+/+
10. P.N.	9	M	10.7	56.2	17.8	31.8	90.4	9.6	53	αα/αα	+/-
11. T.J.	19	F	10.1	59.8	20.3	34.0	86.6	13.4	34	αα/αα	+/+
12. B.N.	27	F	12.4	63.9	22.1	34.5	94.2	5.8	106	αα/αα	+/-

Abbreviations are as defined in Table 1.

(case 2, 3, 4, 6, 7 and 8); although HbF level had decreased, 5 still had HbF levels higher than the normal range. In addition, the levels of HbF determined by alkaline denaturation method were compared with *Xmn I* restriction site at position –158 of Gg gene (Winichagoon *et al*, 1995). Of 76 HbE homozygotes, 40 were homozygous for *Xmn I* (+/+) restriction site with HbF of 1.1-8.7% (mean ± SD = 2.9 ± 2.2), 4 homozygous for absence of *Xmn I* (-/-) restriction site with HbF of 1.0-3.5 % (mean ± SD = 1.6 ± 1.3) and 32 heterozygous for *Xmn I* (+/-) with HbF of 1.2-5.0% (mean ± SD = 2.6 ± 1.2). There was no statistically significant differences in HbF level among the 3 groups.

DISCUSSION

Hemoglobin E (β^E), caused by missense mutation at codon 26, is considered as β^+-thalassemia as the mutation creates a cryptic splicing in codon 25 resulting in reduced β^E-mRNA transcript (Winichagoon *et al*, 1995). In this study, homozygous HbE subjects were asymptomatic, none having hepatosplenomegaly, jaundice or history of transfusion. We have examined the effects of iron deficiency, G6PD deficiency and α-thalassemia (deletion and non-deletion) on the clinical and hematological parameters of HbE homozygotes in order to study the real hematological phenotype of homozygous HbE.

Seven of 76 homozygous HbE individuals were diagnosed as having iron deficiency anemia (serum ferritin less than 16 μg/l) (Hallberg *et al*, 1993). When all 7 subjects were given antiparasitic therapy and iron supplement for 2 months, Hb, MCV and MCH levels were significantly increased. Thus the initial hematological parameters in these 7 homozygous HbE individuals had been affected by their iron deficiency condition. However, for all subjects without iron deficiency anemia given iron supplementation, higher MCH and MCHC values were observed but other hematological parameters did not change. These results indicate that iron supplementation can also affect red blood cell indices in non iron deficient HbE homozygotes.

G6PD deficiency is the most common red blood cell enzymopathy worldwide (Beutler, 1994). The prevalence of G6PD deficiency in 41 male homozygous HbE in this study was 24%, slightly higher than those of previous study (12.08%) using male cord blood (Tanphaichitr *et al*, 1995). G6PD deficiency had no additional adverse effect on hematological parameters of homozygous HbE. In general most G6PD deficient individuals are asymptomatic throughout their life and are unaware of their status. Illness generally manifests as acute hemolysis, which usually arises when red blood cells undergo oxidative stress triggered by agents such as drugs, infection or the ingestion of fava bean (Cocco *et al*, 1998). G6PD deficiency does not seem to affect life expectancy, quality of life or the activity of affected individuals (Hoiberg *et al*, 1981).

We compared cases of homozygous HbE without coinheritance of α-thalassemia and those with affected α-globin genes, including single affected gene ($-\alpha^{3.7}/\alpha\alpha$ and $\alpha^{CS}\alpha / \alpha\alpha$) and two affected genes ($-\alpha^{3.7}/-\alpha^{3.7}$, $-\alpha^{3.7}/ \alpha^{CS}\alpha$ and $--^{SEA}/\alpha\alpha$). We found that no significant difference in hematological parameters between these 2 groups. Other investigators have also studied HbE homozygotes and coinherited α-thalassemia. Overlapping levels of HbE, HbF and other hematological parameters of HbE homozygotes with and without α-thalassemia was observed (Fucharoen *et al*, 2006). We detected 2 cases of heterozygous HbCS ($\alpha^{CS}\alpha/\alpha\alpha$) and 2 cases of compound heterozygous α^+-thal with HbCS ($-\alpha^{3.7}/ \alpha^{CS}\alpha$) in homozygous HbE subjects only by DNA analysis, and not by

HbE electrophoresis. This likely indicates that α^{CS}-globin chains do not form tetrameric Hb molecules with the β^{E}-globin chains (Huisman, 1997). Previously, 2 cases of homozygous HbE with HbH disease (1 case of $--^{SEA}/-\alpha^{3.7}$ and 1 case $--^{SEA}/-\alpha^{4.2}$) with mild anemia were reported and in both cases 100% HbE was present as shown by Hb electrophoresis (Viprakasit and Tanphaichitr, 2004). This may be due to inactive γ-globin expression and leads to misdiagnosis as simple homozygous HbE, since there is no presence of Hb Bart's and F (Viprakasit and Tanphaichitr 2004). It was also observed that red blood cells in all cases of homozygous HbE, with and without affected α-globin gene(s), are mainly target cells. Thus, hemoglobin electrophoresis and hematological parameters are not sufficient enough for the diagnosis of couples at risk for α-thalassemia disease.

The aim of prevention and control program of thalassemia is to prevent babies born with severe thalassemic disease, namely, HbBart's hydrops fetalis, HbE/β^{o}-thalassemia and β-thalassemia major. From this study, we found one case of α^{o}-thalassemia trait out of 76 (1%) homozygous HbE subjects. If the other partner is also α^{o}-thalassemia trait, then the couple will be at risk for having HbBart's hydrops fetalis offspring. We suggest that extensive mutation analysis for α-thalassemia genes in homozygous HbE individuals should be routinely carried out. Moreover, HbE homozygote has twice the risk of having HbE/β-thalassemia offspring compared to HbE heterozygote if the partner has β-thalassemia trait.

Regarding HbF in homozygous HbE subjects studied, 16% (ages 1-27 years) had high percent HbF. Persistence of HbF production is caused by several factors (Rees, 2000). One factor is the persistent expression of γ-globin gene, the molecular mechanism of which is not well understood, and another factor is the presence of several loci, in and outside the β-globin gene cluster. The *Xmn I* restriction site at position –158 of Gγ gene has been strongly correlated with HbF production (Forget, 1998). From our studies we did not find a correlation between *Xmn I* genotype and amount of HbF detected by alkaline denaturation method. However, there were 72 subjects with *Xmn I* +/+ and +/- but only 4 subjects with *Xmn I* -/-. High percent HbF in homozygous HbE may lead to misdiagnosis as HbE/β-thalassemia, which contains HbE and F of 75.4-94.2% and 8.3-24.6%, respectively (Johnson *et al*, 1992). Family studies of parents and sibling or DNA analysis for homozygous hemoglobin E may lead to proper diagnosis.

In summary, homozygous HbE subjects are asymptomatic but carry hidden reproductive risks. Since HbE homozygote carries two abnormal genes, they are twice at risk than heterozygous HbE subject for having HbE/β-thalassemia offspring. Careful studies in couples for β-thalassemia trait is strongly recommended. Hematological data cannot differentiate between homozygous HbE with and without α-thalassemia 1 gene, and thus, for proper counseling management, comprehensive DNA studies of these cases for α-thalassemia 1 are important. If one of the couple has α-thalassemia 1 gene, the partner should also be carefully screened for α-thalassemia gene. Hemoglobin profile and comprehensive DNA analysis for thalassemic hemoglobinopathy in couples at risk planning to have a baby is needed in antenatal clinics in Thailand.

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