

RISK OF A COUPLE HAVING A CHILD WITH SEVERE THALASSEMIA SYNDROME, PREVALENCE IN LOWER NORTHERN THAILAND

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Abstract. Thalassemia screening in pregnant women and their spouses was performed at Buddhachinaraj Provincial Hospital and 8 community hospitals in Phitsanulok; lower northern Thailand. The prevalence of thalassemic carrier state was determined of 1,198 couples. Of these, 4.8% had heterozygous alpha thalassemia-1, 1.6% had heterozygous beta thalassemia, 12.4% had heterozygous hemoglobin (Hb) E, 2.7% had homozygous Hb E and 0.25% of others had abnormal Hb. Eighteen at risk couples (1.5%) were identified. Fifteen couples were at risk for compound heterozygous Hb E / beta thalassemia and the remaining 3 were at risk for homozygous alpha thalassemia-1. Prenatal diagnosis (cordocentesis) was performed in 4 couples at risk, but no fetuses with severe thalassemic disease were detected.

INTRODUCTION

Thalassemia is a leading genetic disease in Thai and Southeast Asia. Patients with beta thalassemia major suffer from chronic anemia, need regular blood transfusions and finally end up with many complications either from transfusion-related or thalassemic disease itself. Fetuses with homozygous alpha thalassemia-1 are stillborn or die shortly after birth posing serious maternal morbidity or even mortality.

The prevalence of thalassemic carrier state differs by different Thai regions. The prevalence of couples at risk for having a child born with severe thalassemic disease also differ for each definite area depending on the different proportions of those carriers. Knowing this epidemiological data should help support the thalassemic prevention and control plan in each area. The prevalence of at risk couples having a child with severe thalas-

semia syndrome in Chiang Mai, upper northern Thailand, is 1.8% (Sanguansersri *et al*, 1998a). We report the prevalence of at risk couples in Phitsanulok, located in the lower part of northern Thailand.

MATERIALS AND METHODS

Thalassemia screening using a simple erythrocyte osmotic fragility test (OF test) (Kattamis *et al*, 1981) was performed on pregnant women and their spouses who attended an antenatal clinic at Buddhachinaraj Provincial Hospital and 8 community hospitals in Phitsanulok, between December 2002 to June 2003. Blood samples with positive results on one of two samples [(+ / -) or (- / +)] or a double positive-result (+ / +) for each of the couples were sent together to the Thalassemia Research Unit, Naresuan University Hospital, Naresuan University for further investigation. Written informed consent was obtained from pregnant women and their spouses before they entered the screening program. Double negative-result (- / -) couples had no risk and needed no further investigations.

Each patient with a positive-OF test blood sample underwent hemoglobin analysis using

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high performance liquid chromatography (HPLC; VARIANT™) (VARIANT,™ 1994) and polymerase chain reaction (PCR) for alpha thalassemia-1 of the Southeast Asian (SEA) type (Chang *et al*, 1991; Kitsirisakul *et al*, 1996). Each patient with a negative-OF test blood sample sent with their spouses with a positive-blood test were screened for heterozygous hemoglobin (Hb) E using microcolumn DEAE Sephadex A50 chromatography, since false negative results on the OF test can occur with heterozygous Hb E (Sanguansermsri *et al*, 1998b).

After matching the results for the pregnant women and their spouses, couples at risk for having a child with severe thalassemia syndrome were identified. These were comprised of homozygous alpha thalassemia-1, homozygous beta thalassemia and compound heterozygous Hb E / beta thalassemia. Prenatal diagnosis using cordocentesis was performed in those at risk pregnant women after genetic counseling. Fetal blood samples were obtained during the 18th to 20th week of gestation. Amidoblack staining of the fetal blood samples was done to exclude maternal blood contamination (Sanguansermsri *et al*, 2001). Hemoglobin analysis of the fetal blood using HPLC was performed for the diagnoses of homozygous alpha thalassemia-1 (Sanguansermsri *et al*, 2001), homozygous beta thalassemia (Sanguansermsri *et al*, 2000) or compound heterozygous Hb E / beta thalassemia (Sanguansermsri *et al*, 2003). Screening and diagnostic tests are shown in Figs 1 and 2.

RESULTS

One thousand two hundred thirty-nine couples were recruited. Forty-one couples were excluded because of incomplete data. There were 1,198 evaluable couples (2,396 persons) left for analysis.

Results of the screening OF test were positive in 611 out of 2,396 persons (25.5%). One hundred five couples were double positive (+ / +) (8.8%), 401 were single positive [(+ / -) or (- / +)] (33.5%) and 692 couples had double negative-results (- / -) (57.8%).

Five hundred six paired blood samples (single and double positive-result couples) were

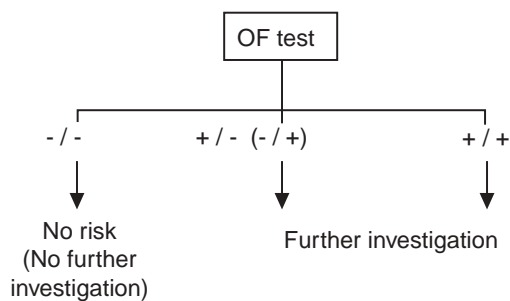


Fig 1—Screening test.

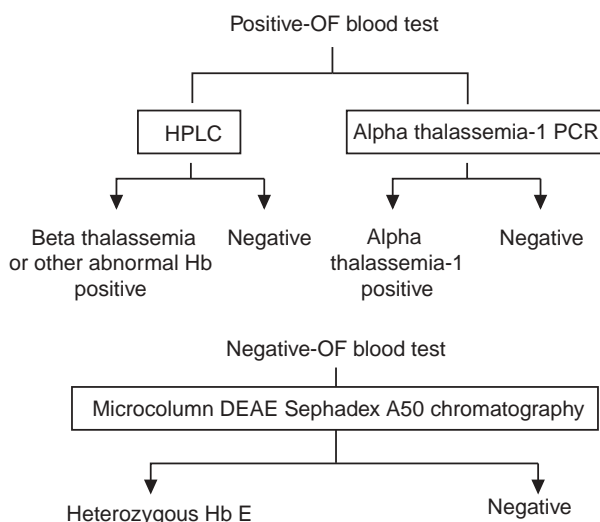


Fig 2—Diagnostic tests (further investigation).

sent for further investigation at the Thalassemia research unit. There were 114 (4.8%) with heterozygous alpha thalassemia-1, 39 (1.6%) with heterozygous beta thalassemia, 298 (12.4%) with heterozygous Hb E, 65 (2.7%) with homozygous Hb E and 6 (0.25%) of heterozygous Hb Constant Spring or other abnormal Hb.

After matching the results for all the pregnant women and their spouses, there were 18 at risk couples (1.5%); 15 at risk for compound heterozygous Hb E / beta thalassemia and 3 at risk for homozygous alpha thalassemia-1 (Table 1). Because of delayed antenatal care, cordocentesis was performed in only 4 at risk couples and no fetuses with severe thalassemic disease were detected.

Table 1
Data of 18 at risk couples.

	Couples	OF test	Hb E screening	Hb A ₂ /Hb E (%)	α thal-1 PCR	Risk	Prenatal diagnosis
1	Husband	Negative	Positive	-	-	Compound heterozygous	Not done
	Wife	Positive	-	4.5	Negative	Hb E / beta thalassemia	
2	Husband	Negative	Positive	-	-	Compound heterozygous	Not done
	Wife	Positive	-	5.2	Negative	Hb E / beta thalassemia	
3	Husband	Positive	-	28.5	Negative	Compound heterozygous	Not done
	Wife	Positive	-	5.3	Negative	Hb E / beta thalassemia	
4	Husband	Positive	-	5.8	Negative	Compound heterozygous	Not done
	Wife	Positive	-	31.4	Negative	Hb E / beta thalassemia	
5	Husband	Positive	-	6.0	Negative	Compound heterozygous	Not done
	Wife	Positive	-	83.4	Negative	Hb E / beta thalassemia	
6	Husband	Positive	-	15.7	Positive	Homozygous alpha	Not done
	Wife	Positive	-	20.5	Positive	thalassemia-1	
7	Husband	Positive	-	88.0	Negative	Compound heterozygous	Not done
	Wife	Positive	-	4.0	Negative	Hb E / beta thalassemia	
8	Husband	Negative	Positive	-	-	Compound heterozygous	Not done
	Wife	Positive	-	4.4	Negative	Hb E / beta thalassemia	
9	Husband	Negative	Positive	-	-	Compound heterozygous	Normal fetal Hb typing
	Wife	Positive	-	5.4	Negative	Hb E / beta thalassemia	
10	Husband	Negative	Positive	-	-	Compound heterozygous	Heterozygous Hb E
	Wife	Positive	-	5.8	Negative	Hb E / beta thalassemia	
11	Husband	Positive	-	84.5	Negative	Compound heterozygous	Not done
	Wife	Positive	-	49.4 ^a	Negative	Hb E / beta thalassemia	
12	Husband	Positive	-	5.3	Negative	Compound heterozygous	Not done
	Wife	Negative	Positive	-	-	Hb E / beta thalassemia	
13	Husband	Positive	-	82.0	Positive	Homozygous alpha	Not done
	Wife	Positive	-	2.4	Positive	thalassemia-1	
14	Husband	Negative	Positive	-	-	Compound heterozygous	Normal fetal Hb typing
	Wife	Positive	-	4.3	Negative	Hb E / beta thalassemia	
15	Husband	Positive	-	4.8	Negative	Compound heterozygous	Not done
	Wife	Positive	-	18.7	Positive	Hb E / beta thalassemia	
16	Husband	Negative	Positive	-	-	Compound heterozygous	Not done
	Wife	Positive	-	48.5 ^a	Negative	Hb E / beta thalassemia	
17	Husband	Positive	-	5.3	Negative	Compound heterozygous	Maternal blood
	Wife	Positive	-	30.8	Negative	Hb E / beta thalassemia	contamination
18	Husband	Positive	-	2.6	Positive	Homozygous alpha	Not done
	Wife	Positive	-	5.8	Positive	thalassemia-1	

^acase of compound heterozygous Hb E / beta thalassemia

DISCUSSION

Because heterozygous Hb E can result in a false negative OF test, single positive-result couples were still at risk for compound heterozygous Hb E / beta thalassemia. Thus after OF tests were done on all couples, we chose to send the blood samples of the couples with single

positive results along with double positive-results to do further investigations.

Since Hb E screening (using microcolumn DEAE Sephadex A50 chromatography) was not performed in double negative-result couples, the prevalence of Hb E was underestimated. Phitsanulok has a high Hb E prevalence com-

pared with other provinces in North Thailand (Pravatmuang *et al*, 1995; Wong *et al*, 2004). Because of the high prevalence of Hb E, compound heterozygous Hb E / beta thalassemia is the major problem in Phitsanulok.

In this prospective study, we found many obstacles to severe thalassemia prevention and control in Phitsanulok, which may represent the state of public health in lower northern Thailand. These obstacles were: low thalassemia screening coverage, delayed antenatal care and lack of knowledge of thalassemia prevention in pregnant women and their spouses. Because therapeutic abortion in severe thalassemic fetuses seems to be the only cost-effective method of thalassemia control, research aiming at thalassemia prevention is worth pursuing to help solve the thalassemia problem in Thais.

ACKNOWLEDGEMENTS

The study was supported by a grant from the Faculty of Medicine, Naresuan University.

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