PRENATAL DIAGNOSIS OF THALASSEMIA IN SONGKLANAGARIND HOSPITAL IN SOUTHERN THAILAND

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Abstract. A thalassemia screening program for pregnant women has been established in Songklanagarind Hospital since 1992. After genetic counseling, a total of 5078 pregnant women accepted entry into a screening program for thalassemia. Couples at risk who should receive prenatal diagnosis were 2.8 %. Total cases who accepted prenatal diagnosis were 135. Total clinical cases were 40 (29.6%) with achievement by prenatal diagnosis of 33 cases (82.5 %). Genetic amniocentesis is the most acceptable method for prenatal diagnosis. Five cases (12.5%) were misdiagnosed due to contamination of maternal blood cells in amniotic fluid cases. Questionable results were reported in 2 cases(5%). Abortion occurred in one case (0.7%). Improvement of surgical technic in prenatal diagnosis reduced the complications and contamination of maternal cells. This program shows the feasibility of prevention and control of thalassemia disease in southern Thailand.

INTRODUCTION

Thalassemia is a common genetic disease in Thailand. Hemoglobin Bart's hydrops fetalis, β-thalassemia/hemoglobin E and hemoglobin Constant Spring (CS) are prevalent (Fucharoen and Winichagoon,1992). Treatment for thalassemia is mainly palliative which is too expensive for a developing country. One strategy for prevention and control of the thalassemia disease is prevention of new births by genetic counseling in combination with prenatal diagnosis. Community control by prospective heterozygote detection, education and fetal diagnosis has already been proven to be greatly outweighed by the financial and social benefits of reducing thalassemia (Angastiniostis and Hadjiminas, 1981; Cao *et al,* 1991.

Genetic counseling, screening programs and prenatal diagnosis of thalassemia in pregnant women have been established in Songklangarind Hospital since 1992, involving the Department of Pathology and Department of Obstetrics and Gynecology . The purpose of this study is to report our experiences from the obstetrics viewpoint. Improvement and development in genetic counseling, surgical technics in prenatal diagnosis and laboratory technics have led to an a program of routine services in our hospital.

MATERIALS AND METHODS

From 1993-1997, a total of 5,078 women accepted entry into a screening program of thalassemia after genetic counseling had been obtained (Panich, 1991). The trained counselors were from the counseling unit. The aim of counseling was to help the couples reach a reasonable and independent decision concerning reproduction . Back-up information such as charts, leaflets and booklets were also used. Hematological screening included measurement of mean corpuscular volume, red cell morphology, one-tube osmotic fragility and DCIP (dichlorophenolindophenol) precipitation test. Standard technics for diagnosis were hemoglobin electrophoresis and DNA analysis (Nopparatana *et al,* 1996). If the pregnant women were carriers, their husbands should have the screening test. These processes took 3-4 weeks for the final results. If both were carriers of severe thalassemia disease, they were informed about the availability of prenatal diagnosis, the risk and accuracy of laboratory diagnosis and concept of selective termination of pregnancy. The couples made their own decisions to accept the prenatal diagnosis.

Genetic amniocentesis is the most acceptable method due to low risks of complications, easier technic and because most patients have late antenatal care (Working Party on Amniocentesis,1978 ; Leschot *et al,* 1985 ; Wanapirak *et al,* 1997). During the first 3 years (1992-1994) we used ultrasound for placental localization and a needle with stiletto gauge no 20 for insertion and aspiration. The contamination of maternal cells was related to failure results. From the following 3 years (1995-1997) until now we developed and improved our technic by:

1. using ultrasound for localization of the site of needle insertion and ultrasound guidance in difficult cases.

2. reducing the size of the needle to gauge no 22-23 (0.7 mm , 90 mm).

3. avoiding placental perforation by:

- postponing amniocentesis for one or two weeks if the placenta covers the whole anterior uterine wall.

- if a transplacental approach is unavoidable, the thinnest area of the placenta, preferably near its margin and away from the cord insertion, should be selected as a site of needleinsertion.

- lubricating inside the needle with heparin to prevent clot formation due to placental perforation or in obese mother.

4. if the placenta is attached posteriorly, needle insertion should be kept near the mid line of the abdomen avoiding the large fetal part and reducing the injury to maternal abdominal wall vessels.

5. discarding first 1-2 ml of amniotic fluid in a separate syringe to avoid maternal cell contamination.

6. scanning after the procedure to check the fetal heart and puncture site, with a view to reassure the mother regading fetal well-being.

The procedure was recorded in detail regarding the location of placenta, the size of needle used, number of needle insertions, the volume and color of amniotic fluid. If the amniocentesis was not successful either due to a failure to obtain fluid or laboratory failure, a repeated amniocentesis was usually performed. All pregnant women were advised to observe abnormal signs and symptoms after the procedure.

Laboratory detection was done by means of the DNA amplifying method, using polymerase chain reaction. The results were usually reported in 10-14 days. All patients were asked to report the outcome of the pregnancy or any other complications. A diagram of the program is shown in Fig 1.

RESULT

From 1993 - 1997 after receiving genetic counseling, a total of 5,078 pregnant women accepted the thalassemia screening program. One hundred and forty couples (2.8%) were found to be carriers for severe thalassemia disease. Only 120 couples (85.7%) decided to proceed to prenatal diagnosis. We also reviewed 15 additional cases of prenatal diagnosis from the year 1992 , so a total of 135 cases were analyzed .

Tables 1, 2 show the patients' characteristic and their hometowns. Most of the patients were farmers and workers (56.3%). About 51.9% had a history of a previous child with severe thalassemia. Referred cases were 22.2%, most of them were from the Phatthalung Province which also had a screening program for thalassemia in pregnant women. Patients came from most provinces in southern Thailand except Phangnga and Ranong. The common types of carriers were β-trait, Hb E trait and α - trait as shown in Table 3. The couples at risk for severe thalassemia diseases such as Hb Bart's hydrops fetalis (homozygous α 1-thalassemia), homozygous β-thalassemia and β-thalassemia/Hb E were counseled to proceed to prenatal diagnosis.

Due to late antenatal care and fewer complications most patients chose amniocentesis as a method of prenatal diagnosis (91.1%) (Table 4) .Chorionic villus sampling, which is a procedure that removes a small sample of chorion and should be done in the first trimester, was performed in only 7.4% of cases. Half of these (3.7%) had to repeat amniocentesis thereafter due to laboratory failure.

One fetal blood sampling (cordocentesis) was performed for confirmation of Hb Bart's hydrops fetalis after amniocentesis. Severe complication from amniocentesis was found in one case (0.7 %), due to amniotic fluid leakage leading to a spontaneous abortion on day 10 after the procedure. No serious complications occurred in the remaining patients within two weeks after the procedure.

Total affected pregnancies were 40 cases (29.6 %). Thirty-three of these (82.5%) were diagnosed as severe thalassemia disease (β-thalassemia/Hb E 21 cases, homozygous β-thalassemia 10 cases, Hb Bart's hydrops fetalis 2 cases). Thirty-two cases chose to terminate the pregnancies. The fetal blood samples were obtained in 11 cases (34.4 %) after abortion, whose results confirmed those of the prenatal diagnosis. One case with the diagnosis of β-thalassemia/Hb E refused to terminate and continued her pregnancy.

Genetic counseling in pregnancy women

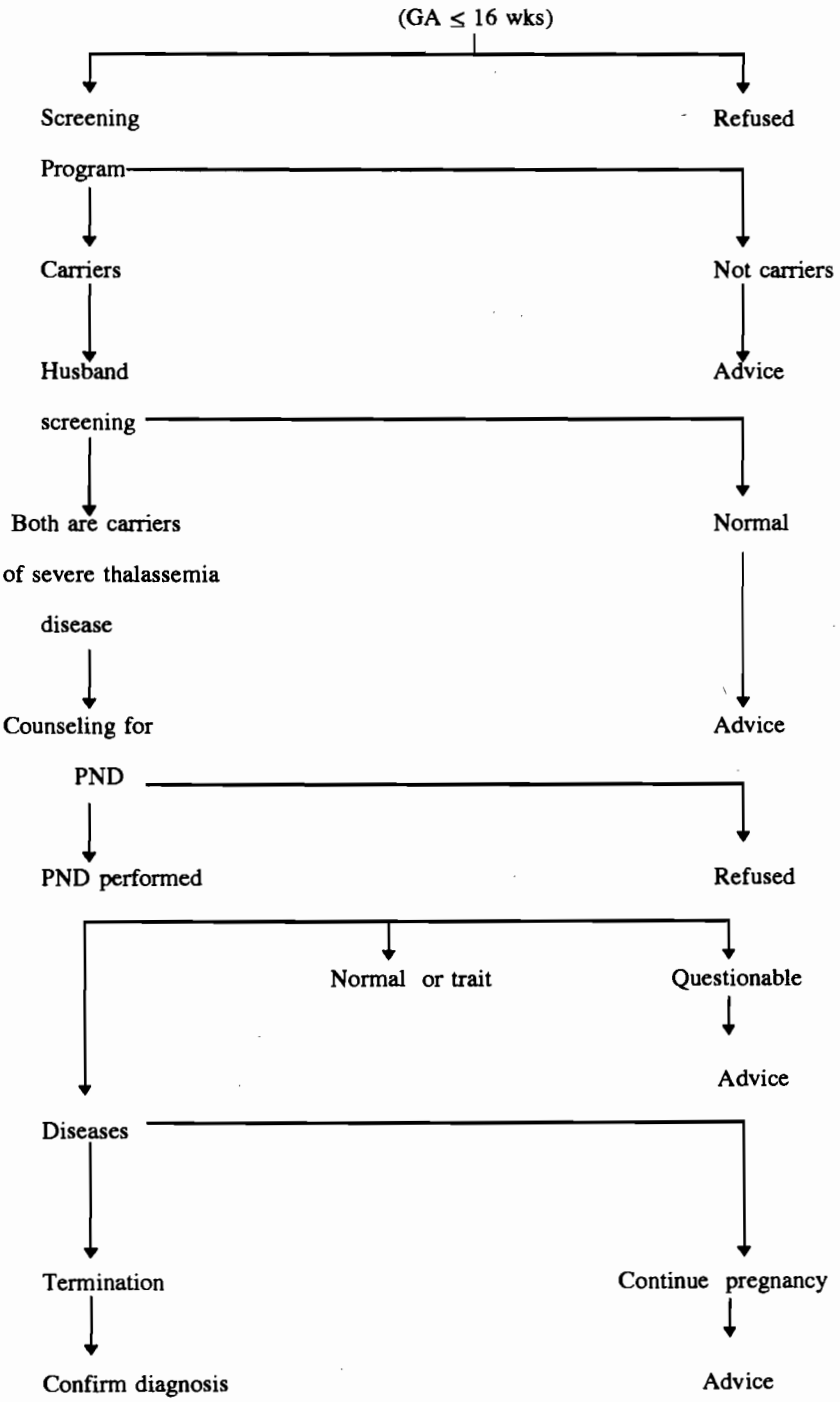

Fig 1–Program of genetic counseling and prenatal diagnosis in pregnant women.

Table 1

Characteristics of 135 patients*.

	No.	%
Occupation		
Housewife	26	19.3
Government officer	17	12.6
Business	14	10.4
Agriculture	53	39.3
Worker	23	17.0
Others	2	1.48
Religion		
Buddhist	127	94.1
Muslim	8	5.9
History of thalassemia	70	52.2
Referred cases	30	22.4
OPD cases	35	25.9

Age - mean = 27.8, SD = 5.3, median = 28, min = 16, max = 41

Table 2

Hometown.

	n=135	%
Songkhla	52	38.8
Phatthalung	33	24.6
Nakhon Si Thammarat	14	10.4
Satun	9	6.7
Trang	5	3.1
Surat Thani	4	3.0
Pattani	4	3.0
Yala	4	3.0
Chumphon	2	1.5
Krabi	2	1.5
Phuket	2	1.5
Narathiwat	2	1.5
Others	1	0.7

Table 3

Hemoglobin typing of wives and husbands.

	Wives		Husbands	
	n=135	%	n=135	%
β-trait	71	52.6	64	47.4
HbE trait	37	27.4	41	30.4
β thal/HbE disease	3	2.2	7	5.2
HbE homozygous	3	2.2	2	1.5
α-trait	24	17.7	16	11.9
HbH disease	-	-	2	1.5
β-α 1 trait	-	-	1	0.7
α1-HbE trait	-	-	1	0.7
Unknown	-	-	1	0.7

Five cases were misdiagnosed. Four cases were related to contamination by maternal cells from the surgical procedure. Three of these were diagnosed as α1-thalassemia whose fetus developed hydrops fetalis later on. One case was prenatally diagnosed as β-thalassemia trait but the child was proven to be β-thalassemia/Hb E after birth. The last misdiagnosed case was from a family which had a previous child with thalassemia disease. The mother was Hb E trait and her husband had normal hematological studies. Amniotic fluid DNA analysis was normal but the child had thalassemic symp-

Table 4

Procedures in patients.

	n=135	%
Amniocentesis	123	91.1
Chorionic villus sampling (CVS)	5	3.7
CVS and amniocentesis	3	3.7
Amniocentesis and cordocentesis	1	0.7

Table 5

Result of prenatal diagnosis.

	n=135	%
1. Normal	24	17.8
2. Trait	61	45.2
β - trait	31	
E - trait	21	
α - trait	9	
3. Diseases	40	29.6
Correct diagnosis	33	24.4
β - thal/Hb E	21	
homozygous β	10	
Bart's hydrops	2	
Misdiagnosis	5	3.7
β - thal/Hb E	1	
Bart's hydrops	3	
unknown	1	
Questionable diagnosis	2	1.5
β - thal/Hb E	1	
Bart's hydrops	1	
4. Questionable diagnosis nornal or trait	10	7.4

toms after birth. Two cases were questionable for disease or trait. One case developed hydrops fetalis at 25 weeks gestation. In the other case it was not possible to determine β gene of the mother: prenatal diagnosis result was Hb X/Hb E. The family chose to terminate the pregnancy: the abortus was β-thalassemia/Hb E disease. All of the 7 problem cases are shown in Table 5.

DISCUSSION

Genetic counseling, hematological screening and prenatal diagnosis of thalassemia disease have been established in Songklanagarind Hospital since 1992. Pregnant women were our target population. The programs were conducted in a prospective way. The percentage of couples at risk of severe thalassemia was relatively high (about 2.8%) in our hospital: an important reason was that half of these had a previous child with severe thalassemia disease. Genetic counseling, hematological screening and prenatal diagnosis were more easily introduced into this group. They were not reluctant to enter into the program. Amniocentesis was the most acceptable method for prenatal diagnosis. It is a safe surgical procedure. Fetal loss rate within two weeks after the procedure was low (0.7%). Risk of spontaneous abortion following amniocentesis has been difficult to estimate accurately because of the lack of randomized trials (Tabor, 1986). However, some authors have mentioned that the risk of spontaneous abortion was increased when associated with blood-stained fluids (from multiple needle insertions with or without placental perforation) and placental perforation (Crane and Kopta, 1984 ; Kappel *et al,* 1987). Misdiagnosis and failure of diagnosis were 17.5 % (7 cases) in our series. All of these cases were contaminated with maternal cells in the amniotic fluid. After 2 - 3 years of experience we improved the surgical technics by avoiding placental perforation, using smaller size of needle and lubricating inside the needle with heparin. These technics can reduce the contamination by maternal cells and improve the final diagnosis. Two cases were unable to be diagnosed prenatally because of laboratory limitation. Improvement in laboratory technics is also very important to identify the mutations of the abnormal genes (Laosombat *et al,* 1995). Therefore genetic counseling should take note of all this information. These programs show the feasibility of prenatal diagnosis for thalassemia in southern Thailand as well as in Bangkok (Fucharoen *et al,* 1991).

ACKNOWLEDGEMENTS

We are grateful to the Counseling unit, Department of Obstetrics and Gynecology, Songklana-

garind Hospital. We also thank Dr Chumnong Nopparatana for his laboratory diagnosis and Professor Vicharn Panich for his encouragement.

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