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Prenatal Diagnosis of Severe Thalassemic Disease at Regional Hospital : 30 Cases Experience at Lampang Hospital

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ABSTRACT

Objective To study prenatal diagnosis of severe thalassemic disease in the aspect of methods of prenatal diagnosis, complications, success rate, results and pregnancy outcomes at a regional Hospital.

Design Descriptive study.

Setting Lampang Hospital.

Subjects 30 pregnant women at risk for severe thalassemic fetus. Prenatal diagnosis were performed in all study subjects at Lampang Hospital, from December 1997 to November 1998.

Methods We chose 2 methods of prenatal diagnosis in our study :- fetal blood sampling and chorionic villi sampling which performed at Lampang Hospital. Fetal villi specimens were sent for DNA analysis by PCR technique and fetal blood specimens were sent for hemoglobin typing by HPLC method at Department of Pediatrics, Faculty of Medicine, Chiang Mai University.

Results Mean maternal age was 26 years and gestational ages were between 14-24 weeks. 27 fetuses were diagnosed prenatally by fetal blood sampling and 3 fetuses by chorionic villi sampling. The results of analysis revealed 6 severe thalassemic fetuses, 20 carriers and 4 normal fetuses. There was no sampling failure. There were 22 livebirths, 1 abortion, 2 dead fetuses in utero at 35 weeks and 38 weeks of gestation and 5 severe thalassemic fetuses in which therapeutic abortion were performed. 28 umbilical cord blood samples were sent for confirmed analysis postnatally and postabortion and all results were correct with prenatal diagnosis.

Conclusion Prenatal diagnosis of thalassemia at Lampang Hospital revealed acceptable complication rates and accurate results. Regional hospitals, therefore, should be able to provide prenatal diagnosis for thalassemic diseases by collaborating with university hospitals

to obtain laboratory assistances. This collaboration will lead to a more effective strategy in controlling this disease in Thailand.

Key words : thalassemia, prenatal diagnosis, regional hospital

Thalassemia is an inherited disease which is among the most prudent public health problems in Thailand. It has been estimated that there are approximately 5,125 newborn babies suffered from severe thalassemic diseases annually. According to the 8th national social and economic development plan (1997-2001), the goal was set to decrease the incidence of thalassemic diseases in neonates by 10 percent. Prenatal diagnosis of severe thalassemic diseases, followed by termination of pregnancy in such cases, is one of the most important strategies in controlling the disease. Prenatal diagnosis of thalassemia, however, does require highly experienced and well qualified physicians to perform chorionic villi sampling, amniocentesis and fetal blood sampling. Moreover, well-equipped laboratory and technical experts are needed to complete the process.[1] Prenatal diagnosis of thalassemia in Thailand, therefore, has been performed successfully only in the university hospital settings. This gives rise to the high expenditure and time-wasting for the couples who are at risk of having a child with severe forms of thalassemia since they have to travel a long distance from their hometowns to one of the university hospitals which are located only in Bangkok and a few other big cities.

Lampang Hospital has provided the screening program for thalassemic carriers in pregnant women since January 1997. With the kind assistances from Department of Pediatrics , Faculty of Medicine, Chiang Mai University, prenatal diagnosis of thalassemia was successfully opened into public services in December of the same year. This study is aimed to investigate prenatal diagnosis of thalassemic diseases at the regional hospital setting in the aspects of methods of diagnosis, complications, success rate, results and pregnancy outcomes.

Materials and Methods

Prenatal diagnosis of thalassemia were performed in 30 pregnant women who were at risk of having a child with one in the three forms of severe thalassemic diseases including hemoglobin Bart's hydrops fetalis, homozygous beta-thalassemia and beta-thalassemia/HbE disease. Informative counseling covering details about the disease, chance of having an affected offspring, and full details of how the diagnostic procedures would be done were provided to all the couples at risk. The decision to undergo prenatal diagnosis was made by the couples after obtaining the full ranges of counseling. Consent forms were collected before performing the procedures.

There are two diagnostic methods carried out in this study which are cordocentesis and transabdominal chorionic villi sampling.

Cordocentesis was performed during the 18 - 24 weeks of gestation. The protocol of this procedure is provided as follow.

1. Ultrasonography was undertaken to assess number of fetus, gestational age, amount of amniotic fluid, structural abnormalities of the fetus, placental implantation site, optimal umbilical cord site for fetal blood sampling and direction of the needle.
2. Providone iodine solution was painted over the selected area of the anterior abdominal wall. Sterile sheet was placed, and the sonographic transducer was covered with sterile plastic bag.
3. Optimal umbilical cord site for fetal blood sampling was reevaluated. Local anaesthetic injection was performed followed by insertion of the 20 guage spinal needle through the anterior abdominal wall to umbilical vessels. The stylet was removed and the spinal needle was, then, connected with 1 ml. heparin-coated syringe. 1 ml. of fetal blood was drawn and transferred into a microtube. The spinal

needle was, then, withdrawn.

4. Bleeding from the cordocentetic site and fetal cardiac movement were evaluated ultrasonographically after withdrawal of the spinal needle. Counseling concerning symptoms that require prompt report to the physicians was provided to the patients before allowing them to return homes.

Fetal blood samples were sent to the Department of Pediatrics, Faculty of Medicine, Chiang Mai University for hemoglobin typing using HPLC.

Transabdominal chorionic villi sampling was performed at gestational age 10 weeks or beyond following the provided protocol.

1. Ultrasonographic evaluation and sterile techniques were undertaken following the previously described steps as in the process of cordocentesis.
2. Optimal placental site for sample collection was evaluated. Local anesthetic injection was applied followed by insertion of the 20 guage spinal needle through the anterior abdominal wall to the placenta in the parallel direction to the placental implantation plane. Stylet was removed and the spinal needle was, then, connected with 20 ml. syringe containing 5 ml. of 0.9% NSS. Continuous aspiration was applied through the syringe while moving the spinal needle up and down the whole length of placenta 3-4 times before withdrawal of the spinal needle.
3. After withdrawal of the spinal needle, careful evaluation was performed ultrasonographically to determine whether there was hematoma presented at the placental sampling site, or if there were any kinds of abnormal fetal cardiac movements appeared. Full details of symptoms that warrant prompt report to the physicians were given to the patients before allowing them to return homes.
4. Chorionic villi were collected in the test tube containing 0.9% NSS. and sent to Department of Pediatrics, Faculty of Medicine, Chiang Mai University for DNA analysis.

The results of both chorionic villi sampling and fetal blood sampling would normally be informed within 2 weeks after specimen collection. The pregnant women involved in this study were followed until the ends of their pregnancies. After pregnancy termination, umbilical cord blood samples were collected for hemoglobin typing or DNA analysis to confirm the results of prenatal diagnosis.

Results

During the study period (1 December 1997 - 30 November 1998), there were 30 pregnant women at risk of having fetuses with severe thalassemic diseases. Mean maternal age was 26 years and most of the women studied were multiparous. There were 30 fetuses being investigated in this study, 15 fetuses were at risk of having hemoglobin Bart's while the other 13 and 2 fetuses were at risk of having beta-thalassemia/HbE disease and homozygous beta-thalassemia, respectively. Cordocentesis were performed in 27 fetuses while 3 fetuses were diagnosed by chorionic villi sampling (Table 1).

[There was one pregnant woman who presented with dizygotic twins, in which each of the twin fetuses was diagnosed by fetal blood sampling. Besides this, there was another study subject in which both fetal blood sampling and chorionic villi sampling were performed to confirm the diagnosis of hemoglobin Bart's hydrops fetalis.]

The mean duration of sample collection were 22 minutes, and 3 minutes in cordocentesis and chorionic villi sampling, respectively. There was no sampling failure occurring in this study. Mean gestational age at which the procedures were performed were 20.7, and 14 weeks in fetal blood sampling and chorionic villi sampling, respectively (Table 2). 96.3% of cordocentesis were performed at free umbilical loop. Results of this study revealed 4 normal fetuses, 20 fetuses that were carriers for thalassemia, 3 fetuses with hemoglobin Bart's hydrops fetalis and 3 fetuses with beta-thalassemia/HbE disease (Table 1). There was one abortion occurring 3 days after fetal blood sampling. In that unfortunate case, cordocentesis was attempted only once and the whole procedure was finished in 20 minutes. Result of the blood sample study revealed

that the abortus was a carrier of beta-thalassemia.

Surprisingly, there was one couple who decided to continue pregnancy eventhough the result of prenatal diagnosis turned out that their fetus was the one with beta-thalassemia/HbE disease.

Pregnancy outcomes revealed 22 livebirths,1 abortion, 2 dead fetuses in utero at 35 weeks and 38 weeks of gestation, and the other five pregnancies were terminated as a result of having fetuses with severe thalassemic diseases (Table 3). In the cases of dead fetus in utero, women were diagnosed gestational diabetes, postnatal.

After pregnancy termination, umbilical cord blood samples were collected in 28 cases for confirmed analysis, and all the postnatal or postabortion results were compatible with those of prenatal diagnosis. Confirmatory evaluation was not performed in 2 cases since in one case the umbilical blood sample collected was too few to analyse, and in the other case the birth took place in another hospital.

Mean weight and gestational age of the fetuses at birth were 2,845 g. (Table 4), and 38.2 weeks (Table 5), respectively. 16 fetuses were delivered vaginally (66.7%), 5 fetuses were performed cesarean section (20.8), while in the other two (8.3%) and one (4.2%) fetuses, vacuum extraction and breech assisting were performed, respectively.

Table 1. Prenatal diagnosis for severe thalassemic diseases

At risk / Data	Hb Bart's hydrops fetalis	Beta thalassemia /Hb E disease	Homozygous beta- thalassemia	Total
1. No. of fetuses. (cases)	15	13	2	30
2. Diagnostic methods.				
2.1 Cordocentesis	12	13	2	27
2.2 TA-CVS	3	-	-	3
3. Complications.				
3.1 Abortion	-	1	-	1
4. Analysis techniques.				
4.1 DNA analysis	3	-	-	3
4.2 Hemoglobin typing	12	13	2	27
5. Results of PND.				
5.1 Normal	3	1	-	4
5.2 Alpha-thalassemia 1 trait	9	2	-	11
5.3 Beta-thalassemia trait	-	5	2	7
5.4 Hb E trait	-	2	-	2
5.5 Hb Bart's hydrops fetalis	3	-	-	3
5.6 Beta-thalassemia/Hb E disease	-	3	-	3

NB. 1. TA-CVS : Tranabdominal chorionic villi sampling.
2. PND : Prenatal diagnosis.

Table 2. Gestational ages at cordocentesis

Gestational ages (weeks)	No. of cases
14 *	3
19	1
20	9
21	15
22	1
24	1
total	30

* Gestational age at CVS

Table 3. Pregnancy outcome

Pregnancy outcome.	No. of cases
1. Livebirths	21
2. Livebirths at other hospital	1
3. Abortion	1
4. Dead fetus in utero	2
5. Termination of affected pregnancy	5
total	30

Table 4. Birthweights

Birthweights(grams)	No. of cases
< 1,999	1
2,000 - 2,499	5
2,500 - 2,999	8
3,000 - 3,499	7
> 3,500	3
total	24

Table 5. Gestational ages at delivery

Gestational ages at delivery (weeks)	No. of cases
< 37	3
37-41	20
> 42	1
total	24

Discussion

Most of the pregnant women who attended the antenatal care clinic at Lampang Hospital normally presented themselves to the physicians during the second trimester of their pregnancies. After the initial blood evaluation, a few weeks is required in order to know which couples are at risk of having fetuses with severe thalassemic diseases. Fetal blood sampling, therefore, was normally chosen to be a diagnostic procedure of choice since the gestational ages of the couples at risk were optimal for this procedure to be undertaken (18-22 week gestation). Only in a few cases who presented to the antenatal care clinic in their first trimester of gestation, chorionic villi sampling were performed at 10-14 week gestation.

This study showed that it took approximately 22 minutes to complete the whole procedure of cordocentesis (from insertion of the needle through the anterior abdominal wall to obtaining blood sample). This is relatively slow, compared to the 13 minute duration of the same procedure reported by Wanapirak, et al.[2] The lack of the physician's experience might contribute to this rather long procedure.

However, the procedure tends to require less time as the physician gains more experiences. Another possible factor that might adversely affect the procedure is the relatively poor quality of the ultrasonographic instruments, and hence the poor quality of the pictures obtained. This, sometimes, resulted the necessity to change the insertion site. However, there were no more than 2 insertional attempts being done in this study since it is generally accepted that the abortion rate is increased with the number of insertional attempts.[3,4]

There are wide ranges of complications that could arise after fetal blood sampling including abortion, fetal death in utero, fetal bradycardia, bleeding from umbilical cord, hematoma at site of blood sampling, abruptio placentae, and chorioamnionitis.[5] In this study we found that there was 1 abortion (3.3%) which was quite comparable to what has been reported by a large scale study of Ghidini, et al,[6] which demonstrated that abortion rate of the procedure was 0.8-4.3%. The abortion rate presented in this study, however, might not represent the exact rate since the number of cases being investigated in this study was quite low. Further study, therefore, is required before the more precise view about the complications of fetal blood sampling can be properly proposed.

We found one case of dizygotic twins of which the result of fetal blood evaluation in one fetal could differ from that of the other. We, thus, performed fetal blood sampling from both fetuses and sent samples for further evaluation separately. The results in this case revealed that both fetuses were carriers for alpha-thalassemia.

We were at the beginning stage of assessing chorionic villi samples by DNA analysis and, thus, only able to diagnose fetuses at risk of having hemoglobin Bart's hydrops fetalis. Fetal blood sampling or serial ultrasonographic evaluations are also required to confirm the results of DNA analysis. In our study, there was 1 fetus being diagnosed of having hemoglobin Bart's hydrops fetalis from chorionic villi evaluation. Result from fetal blood sampling of this case at 20 weeks of gestation confirmed the diagnosis. Termination of pregnancy was, then, commenced. Other two fetuses were diagnosed of being alpha-thalassemia carriers from chorionic villi evaluation. Serial ultrasonographic assessments at monthly interval did not show any signs of hemoglobin Bart's hydrops fetalis.

To confirm the results of prenatal diagnosis, we performed postnatal or postabortion umbilical cord blood analysis in 28 cases being studied (93.3%). All of the postnatal or postabortal analysis confirmed the results of prenatal diagnosis.

In conclusion prenatal diagnosis of thalassemia at Lampang Hospital revealed acceptable complication rates and accurate results. Regional hospitals, therefore, should be able to provide prenatal diagnosis for thalassemic diseases by collaborating with university hospitals to obtain laboratory assistances. This collaboration will lead to a more effective strategy in controlling this disease in Thailand.

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