Prenatal Diagnosis of Thalassemia and Hemoglobinopathies: 20 Years Experience at Siriraj Hospital

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ABSTRACT

Objective: To show the experience of prenatal diagnosis of Thalassemia and hemoglobinopathies in Siriraj Hospital.
Methods: Hb Bart's hydrops fetalis can be detected by DNA study from polymerase chain reaction (PCR) product in the first trimester of pregnancy either by chorionic villus sampling (CVS) or aminocentesis but in late pregnancy it can be detected unambiguous by ultrasonography at 18-20 weeks gestation, the suspected cases are confirmed by fetal blood sampling and Hb electrophoresis. Prenatal diagnosis (PND) for β-thalassemia diseases can be done at early pregnancy by direct visualization of the PCR products on electrophoresis or by dot blot analysis of amplified DNA with a set of HRP-labeled oligonucleotide probes complementary to the mutations. If the mutation is unknown. The couples have to wait for Hb analysis by HPLC or in vitro globins chain analysis from fetal blood in the second trimester.
Results: The results of PND at Siriraj Hospital are summarized as Hb Bart's Hydrops fetalis 228 cases, Homozygous Beta-Thalassemia 126 cases, and Beta Thalassemia/Hb E disease 550 cases. There are various methods of sampling namely chorionic villous sampling, amniocentesis, fetal blood sampling, ultrasound, or even combined method. There are minimal incidences of fetal loss 9 out 904 cases which, comparatively give us one of the best center for prenatal diagnosis in Asia.
Conclusion: Of the 904 pregnancies, the diagnosis were obtained in 891 pregnancies in which had 5 fetal loss from dead fetus in utero after fetal blood sampling in the second trimester. The other complication occurred after sampling failure.

Keywords: Amniocentesis; chorionic villus sampling (CVS); fetal blood sampling (FBS); HPLC; PCR; prenatal diagnosis (PND); ultrasound (U/S); thalassemia

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Thalassemia in one of the most common single gene disorders, a group of autosomal recessive inheritants, found in the peoples of Mediterranean, the Middle East and Southeast Asia.[1,2,3]

In Thailand the genes frequencies of α - thalassemia are 20-30 percent, β - thalassemia gene frequencies vary form 3 to 9 percent. Haemoglobin (Hb) E from 10-50 percent and Hb constant spring 1- 8 percent. The four major thalassemia diseases are Hb Bart's hydrops fetalis, (homozygous α thalassemia 1), Cooley's anemia (homozygous β - thalassemia), β thalassemia / HbE and Hb H disease.

Over 12,000 of the annual one million newborns are expected to be affected with this disorder.[3] About 5,000 newborns are thalassemia major which include homozygous β - thalassemia and β thalassemia / Hb E.

Homozygous α thalassemia 1 leads to a totally lethal Hb Bart's hydrops fetalis which die in utero or few minutes after birth, and their mothers often suffer from obstetric

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complications such as pre-eclampsia, antepartum or post-partum hemorrhage.

Prevention and control of thalassemia require a well-planned program of population screening and genetic counseling. Prevention of new births of thalassemias in the couple at risk can be implemented by prenatal diagnosis (PND) with selective abortion of the affected fetus.

The procedure for fetal cell sampling and the method of analysis depends on the gestational age of the pregnancy and gene mutations known or not.

PND by using fetal DNA isolated from either the amniotic fluid fibroblasts in the second trimester amniocentesis or chorionic villi sampled in the first trimester of pregnancy has enabled DNA study by polymerase chain reaction (PCR).

Assaying the Hb type of the pure fetal blood obtained by cordocentesis under ultrasound guidance is an alternative option when DNA studies are inconclusive or for those who present relatively late in pregnancy.

In this study we performed PND using our 20 years experience to show how changes in method have been associated with a decrease in the obstetric risk of the procedure.

MATERIALS AND METHODS

From 1985 we started to do the PND for thalassemia by fetal blood sampling which was initially obtained by blind placental needling (placentocentesis)[4,5] and transferred to fetoscopic blood sampling for the next years after we purchased the Olympus Fetoscope.

Improvements in ultrasound resolution led to direct needling of the umbilical cord vessel guided by ultrasound (Cordocentesis)[6] which replaced the fetoscopic blood sampling after the first 3 years of PND in Siriraj Hospital. A blood sample obtained from needling at the cord insertion or floating part gives a better yield of pure fetal blood with minimal or no contamination by maternal red cells.

Cordocentesis has been standard for fetal blood sampling from 1990 up to now, and the hemoglobins analysis by an automated high - performance liquid chromatography (HPLC) system[7] or in vitro globin chains analysis for laboratory diagnosis.

The introduction of the polymerase chain reaction (PCR) has enabled us to perform early PND by using fetal DNA isolated from either the amniotic fluid fibroblasts or chorionic villi in the first trimester of pregnancy. CVS can be performed transcervically or transabdominally under ultrasound guidance. Initially we do the CVS by using a 16-gauge Medicut cannula or a Portex cannula transvaginal guided by U/S with suction provided by a 10 ml. Syringe.[8,12]

Of the four failures, one pregnancy was lost as a result of infection, so we decided to change CVS to transabdominally by using the 18 or 21 gauged spinal neddle guided by ultrasound.

Homozygous α - thalassemia (Hb Bart's hydrops fetalis) can be diagnosed unambiguously ultrasonography by the presence of hydrothorax, pericardial effusion and anasarca.

The early signs of prehydrops include cardiomegaly, dilatation of the umbilical vein and placentomegaly[9] can be detected by Ultrasound. The suspected case was confirmed by hemoglobin electrophoresis of the fetal blood obtained from cordocentesis.

A total 904 pregnant women were at risk of having fetuses of thalassemia diseases Hb Bart's hydrops fetalis (228), homozygous β thalassemia (126) and β - thalassemia / HbE disease (550).

TABLE 1. Indications for Prenatal diagnosis.

Prenatal diagnosis	1985 - 2005
b-thal/Hb E disease	550 (60 %)
Hb Bart's hydrops fetalis	228 (26 %)
Homozygous b-thalassemia	122 (14 %)
Total	904 (100 %)

The procedure for fetal cell sampling and the method of analysis depends mainly on the gestation of pregnancy. CVS (380) and amniocentesis (7) provided fetal DNA for direct visualization of the PCR product on electrophoresis or by dot blot analysis of amplified DNA with a set of HRP - labeled nucleotide probes complementary to the mutation.[10]

If the mutation is unknown or someone presents relatively late in pregnancy fetal blood sampling (448) for Hb analysis is by HPLC or in vitro globins chain analysis.

Combined or repeated PND (57) is needed when either the original sample was inadequate for diagnosis, or when there was an ambiguous result. Most of the repeated cases are diagnosed by cordocentesis.

RESULTS

Three major thalassemia diseases indicated for prenatal diagnosis of homozygous α - thalassemia 228 (26%), the couples at risk of having homozygous β thalassemia 126 (14%) and beta thalassemia / Hb E 550(60%) are shown in Table 1.

Most cases are the couple who had given birth to a previously affected child prior to the screening program for thalassemia in pregnancy which was established in Siriraj Hospital in the year of 2000.

Table 2 shows the first case in Thailand was prenatal diagnosis of the fetus at risk for β - thalassemia /HbE disease by placentocentesis. Of the fetal blood sent for globin chain analysis[4] only 5 cases were done by this method, with one fetal loss from amniitis.

Thirteen cases of fetoscopic fetal blood samples were transferred from placentocentesis with a better yield of pure fetal blood. The largest problem occurred among patients where the amniotic fluid became heavily blood stained as a result of the procedure. In two cases the diagnosis could not be achieved because of sampling failure and one case had amniotic fluid leakage with abortion.

Three cases of fetal loss were from amniotic leakage, amniitis and intrauterine death due to placental or fetal trauma.

Cordocentesis is the only procedure for fetal blood sampling in the last 15 years with the diagnosis not achieved from sampling failure decreased from 3/18 to 4/430 comparing with placentocentesis and fetoscopic fetal blood sampling.

TABLE 2. Methods of fetal sampling.

Fetal sampling	1985 - 1990	1991 - 2005
Fetal blood sampling	50	398
Placentocentesis	5	-
Fetoscope	13	-
Cordocentesis	32	398
CVS	12	368
Amniocentesis	5	2
U/S	12	-
Combined	2	55
Total	75	829

TABLE 3. Summary of prenatal diagnosis for Hb Bart's hydrops fetalis, homozygous b-thal, b-thal/Hb E at Siriraj Hospital. From 1985 to 2005).

High-risk	Hb Bart's Hydrops Fetalist	Homozygous Beta - Thalassemia	Beta - Thalassemia/ HbE disease	Total
No. of pregnancy	228 (26%)	126 (14%)	550 (60%)	904 (100%)
Method of sampling				
CVS	103	48	229	380
Amniocentesis	3	1	3	7
Fetal blood sampling	89	69	290	448
U/S	12	-	-	12
Combined method	21	7	29	57
No. of Dx achieved	227	125	539	891 (98.5%)
No. of Dx not achieved	1	1	11	13 (1.5%)
Result				
Unaffected	143	107	409	659
Affected	85 (37%)	18 (15%)	129 (24%)	232 (26%)
Fetal Loss	2	1	7	10 (1%)
Misdiagnosis of the disease	3	2	2	7

Hb Bart's hydrops fetalis can be diagnosed by ultrasound. We used ultrasound scanning to monitor the fetuses in 9 pregnant women who had previously delivered infants with Hb Bart's hydrops fetal, two cases were suspected to be affected fetuses at 20 weeks' gestation because of cardiomegaly, dilatation of the umbilical vein (>8mm.) and increased placental thickness (>5 cm). The diagnosis was confirmed by hemoglobin electrophoresis of the fetal blood obtained from cordocentesis quided by ultrasound.

For chorionic villus sampling, initially we do the PND by transvaginal chorionic villus sampling by Portex catheter guided by ultrasound.

Of the four failures from procedures one pregnancy was lost as a result of amniotic fluid leakage and infection, but after we changed to transabdominal CVS the fetal loss, decreased to 1 percent.

The results of PND in Siriraj Hospital are summarized in Table III. Of the 904 pregnancies the diagnoses were obtained in 891 pregnancies (98.57) in which 7 had a dead fetus in utero immediately after fetal blood sampling in the second trimester.

The other 3 complications occurred ofter sampling failure amniitis 1, dry amniotic fluid 1, and spontaneous abortion 10 days after transvaginal chorion biopsy 1.

Misdiagnosis of the thalassemia disease were found in 3 cases at risk for Hb Bart's hydrops fetalis and 2 cases each of homozygous β - thalassemia and β - thalassemia / HbE disease.

DISCUSSION

Prenatal diagnosis of thalassemia and other hemoglobinopathies in Thailand have been carried out since 1985 by placentocentesis with globin chain synthesis and chromatography[4] with the high contamination of maternal blood made difficult for laboratory diagnosis. The fetoscopic blood sampling superseded placentocentesis which required a certain expertise with a fetal loss rate of approximately 5%[5] and some conditions such as placenta inserted anteriorly, oligohydramios were contraindicated for fetoscopy.

Fetal blood may be obtained by needling from the umbilical cord vessel guided by ultrasound (Cordocentesis).[6] The technique is simple and easy to train, and gives a better yield of pure fetal blood with minimal or no contamination by maternal red cells. Fetal blood can be obtained at 18 or more weeks gestation using a 20 gauge spinal needle, so nearly all cases can be done by cordocentasis.

The serious complications occur in less than 2% of cases. Percutaneous umbilical cord blood sampling (Cordocentesis) has been a standard technique for fetal blood sampling since 15 ago.

Hb Bart's hydrops fetalis can be detected unambiguously by ultrasonography at 18 - 20 weeks. gestations[9] which is beneficial for screening in the case of late pregnancy, The suspected cases by U/S are confirmed by fetal blood sampling and hemoglobin electro phoresis.

The introduction of the polymerase chain reaction (PCR) has enabled us to perform early PND by using fetal DNA isolated from either the amniotic fluid fibroblasts or chorionic villi by CVS. in the first trimester of pregnancy.[11]

Chorion biopsy has been taken by a variety of methods included blind aspiration,[11] endoscopic direct vision biopsy, ultrasound guided aspiration or biopsy forceps.[8,12,13]

Transvaginal CVS using a 1.5 mm. diameter plastic catheter with an aluminium obturater (Portex) guided by real - time ultrasound had been used in the first 5 years of PND, but infection and amniotic fluid leakage were major problems. Transabdominal chorionic villus sampling can avoid infection and AF leakage.

Chorionic villus sampling is appropiate for the place where DNA study is available. This procedure is expensive and requires technical skill, preventing its wide use, especially in developing countries.

Cordocentesis provides pure fetal blood for assaying the Hb typing by automated HPLC and is a suitable option when DNA studies are inconclusive or for those who attend the anternatal clinic relatively late in pregnancy.

With the current technology for PND the obstetrician has to sample cord blood or chorion from the placenta for further analysis which is an invasive procedure and may result in certain complications such as infection or abortion.

PND by using fetal nucleated red cells in the maternal circulation has been developed[14] and it may replace the current conventional technique in the future.

Preimplantation diagnosis of thalassemia[15] has been developed which may be suitable for an individual family, but not for a prevention and control program.

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