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Ultrasound Measurement of Placental Thickness to Detect Pregnancies Affected by Homozygous α - Thalassemia-1

Pasagon Pakary MD,
Teera Wacharaprechanont MD,
Waralax Yamasamit MD,
Kitti Wongkittisophon MD,
Dhiraphongs Charoenvidhya MD.

Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

ABSTRACT

Objective To evaluate the efficacy of ultrasound measurement of placental thickness (PT) at 16 - 22 week's to determine fetal homozygous alpha-thalassemia1 before the appearance of Hb Bart's hydrops fetalis.

Design Retrospective and prospective descriptive study.

Setting Maternal-fetal medicine Unit, King Chulalongkorn Memorial Hospital, Bangkok, Thailand.

Subjects 216 pregnant women at risk for having a fetus with Hb Bart's disease attending the antenatal clinic at King Chulalongkorn Memorial Hospital, were recruited to the study from June 2000 to May 2003.

Method We measured placental thickness by ultrasound at 16-22 weeks' gestation in 216 at-risk pregnant. Pregnancies at risk were examined serially by ultrasound to measure the placental thickness and other features of hydropic fetuses. Cordocentesis for hemoglobin study was done to confirm the diagnosis of Bart's hydrops fetalis. Unaffected fetuses were followed up by serial ultrasound scanning every 4 weeks until the 28th weeks of gestation, also all unaffected fetuses were confirmed after birth. The sensitivity and specificity with 95% CI were calculated for various cut-off levels of placental thickness (ROC curve) for different gestational ages.

Results The sensitivity and specificity in detecting affected pregnancies, at 16-18 week's gestation at cut-off 3.22 cm placental thickness by ROC curve were 0.95 (0.89-1) and specificity 0.96 (0.93-0.99). At 18-20 weeks, at cut-off 3.56 cm. the figures were 0.90 (0.59-0.98) and 0.96 (0.70-0.98), respectively. At 20-22 week's gestation at cut-off 3.85 cm sensitivity and specificity were 0.90 (0.59-0.98) 0.95 (0.70-0.98), respectively.

Conclusion On the basis of the receiver operating characteristic curve using those cut off values of placental thickness measurement has very high accuracy in predicting homozygous alpha-thalassemic fetuses. The placental thickness sonographic parameter should be considered to reduce the number of the invasive procedures in dianosis of Bart's hydrops fetalis.

Key words: Hemoglobin (Hb) Bart's hydrops fetalis, Placental thickness, Thalassemia, Ultrasound, homozygous alpha-thalassemia-1.

α-Thalassaemia is a common genetic abnormality. Couples with heterozygous α-thalassaemia-1 face a 25 percent risk of having a fetus with homozygous α- talassaemia-1. Homozygous α-thalassaemia-1 is the commonest cause of hydrops fetalis in South-East Asia.[1-5] The hydropic fetus has cardiomegaly, pericardial effusion, pleural effusion, ascites, hepatomegaly and a large edematous placenta. The fetus is either stillborn or dies soon after birth. Despite the inevitable outcome, prenatal diagnosis is necessary because the pregnancies are often complicated by pre-eclampsia and hemorrhage with significant maternal morbidity.

Prenatal diagnosis is usually performed by DNA analysis or hemoglobin analysis with HPLC. Chorionic villi or amniocytes obtained from chorionic villous biopsy or amniocentesis are needed for DNA diagnosis method. Cordocentesis for fetal hemoglobin analysis is needed for hemoglobin ananlysis method. However these techniques are expensive or invasive and require an experienced operator. Also these procedures have significant risk of fetal loss. Ultrasonographic features of hydrops fetalis, i.e., subcutaneous edema, pleural effusion, pericardial effusion, ascites, etc., were reported to be apparent in affected pregnancies only after 20 weeks of gestation.[6-8,11-13] With the advent of high resolution ultrasound equipment, these changes would already be evident in one third of the affected pregnancies at 17-18 weeks and could be seen as early as in the 12th weeks.[12-13] Pregnancies at risk may then be examined serially by ultrasound to detect these changes, followed by cordocentesis and hemoglobin study for confirmation of the diagnosis. This prenatal diagnostic approach, however, is not a sensitive test for homozygous α- thalassaemia-1 in the early stage of pregnancy. Two-third of the affected pregnancies would be missed on ultrasound examination before 20 weeks of gestation.[12-14] Other subtle ultrasonographic signs of fetal anemia such as fetal cardiomegaly, pericardial effusion and placentomegaly may be more useful in the prediction of disease in early gestation.

Because of high cost or unavailability of DNA analysis in our country, we developed a model of a limited budget prenatal diagnosis of α-thalassaemia-1 disease. These methods included prospectively universal screening of couple at risk of α-thalassaemia-1 trait, serial ultrasound from 16-28 weeks gestation and follow up after birth. Couple at risk of α-thalassaemia-1 trait was identified by having low mean cell volume (MCV) and mean corpuscular hemoglobin (MCH) and normal HbA2 level in both partners. Confirmation of α-thalassaemia-1 trait could only be diagnosed by DNA analysis. Because most of our patients could not afford the high cost of this molecular diagnosis, we recruited all suspected cases for serial ultrasound screening for fetal anemic and hydropic signs starting from 16-28 weeks gestation at frequency of 4-weeks-interval. Subtle ultrasonographic signs of fetal anemia (fetal cardiomegaly, pericardial effusion and placentomegaly) were considered to be fetus at risk of Hb Bart's disease and cordocentesis for hemoglobin study with HPLC would be performed at 18-22 weeks to confirm the diagnosis. Unaffected sonographically normal fetuses will be followed monthly until 28 weeks pregnancy by serial ultrasound searching for ultrasonographic features of hydrops fetalis, i.e., subcutaneous edema, pleural effusion, pericardial effusion, ascites. All sonographically normal fetuses would then be confirmed after birth.

The objective of this study is to evaluate the efficacy of ultrasound measurement of placental thickness (PT) at 16-22 week's to determine fetal homozygous α-thalassaemia-1 before the appearance of Hb Bart's hydrops fetails. We investigated the placental sonogram in these at risk fetuses in the early second trimester with the routine use of obstetric ultrasound at 16-22 week's gestation. This report may be a useful aid in the selection of high-risk pregnancies for further confirmation.

Material and Method

This is study was conducted as a retrospective and prospective descriptive study at Maternal-Fetal Medicine unit since 2000. Couples at risk of α-thalassaemia-1 trait attending the antenatal clinic at

King Chulalongkorn Memorial Hospital were recruited to the study from June 2000 to May 2003. Informed consent was obtained from each participant.

Carrier identification was performed by electronic measurement of mean cell volume (MCV) and mean corpuscular hemoglobin (MCH). Individuals found to have a low HbA2 level is elevated (<27pg) were then investigated by estimating the HbA2 level. If the HbA2 level is elevated (>3.5%), then beta-thalassemia is indicated. Unexplained reduced MCV and MCH levels with normal HbA2 level of both partners were our indicatives of α-thalassaemia-1 trait. At-risk couples who could not afford DNA confirmation were recruited for serial ultrasound screening starting from 16-28 weeks gestation at frequency of 4-weeks-interval. Subtle ultrasonographic signs of fetal anemia (fetal cardiomegaly, pericardial effusion and placentomegaly) were considered to be fetus at risk of Hb Bart's disease and cordocentesis for hemoglobin study with HPLC would be performed at 18-22 weeks to confirm the diagnosis. Unaffected sonographically normal fetuses will be followed monthly until 28 weeks pregnancy by serial ultrasound searching for ultrasonographic features of hydrops fetails, i.e., subcutaneous edema, pleural effusion, pericardial effusion, ascites. All sonograghically normal fetuses would then be confirmed after birth.

All measurements were performed with an Aloka model. Model 5000 (Aloka Co., Ltd., Tokyo, Japan) Ultrasound machine with a 3.5 MHZ abdominal transducer. The transducer was oriented perpendicularly to the placenta and placental thickness was measured at the center; vertical scanning through the thickest portion of the placental was used and avoid measuring at the focal uterine contraction site or in the fundal or lateral position where the placenta may be folded to minimize measurement error.

216 couples with heterozygous α-thalassaemia-1 were studied. They were at risk of giving birth to infants with homozygous α-thalassaemia-1 and were examined between 16 to 22 weeks gestation for prenatal diagnosis. Their gestational age was calculated from last menstrual period and confirmed by ultrasound measurement of biparietal diameter and femur length.

We measured placental thickness by ultrasound at 16-22 weeks gestation in 216 at risk pregnant women. Pregnancies at risk were examined serially by ultrasound to measure the placental thickness and other features of hydropic fetuses i.e., subcutaneous edema, pleural effusion, pericardial effusion, ascites were observed. Cordocentesis for hemogloblin study was done to confirm the diagnosis of Bart's hydrops Fetalis (positive cases). Unaffected fetuses were followed up by serial ultrasound scanning every 4 weeks until the 28th week of gestation. Also all unaffected fetuses were confirmed after birth. Finding at first ultrasound examination were used. Placental thickness values were grouped in 2 weeks period from 16-22 weeks' gestation which the numbers of pregnancies studied in 16-18 weeks, 18-20 weeks and 20-22 weeks subgroups were 73, 108, and 45 respectively. Positive values were calculated from the positive cases. Negative values were calculated from the pregnancies with subsequent normal outcome. The sensitivity and specificity with 95% confidence interval were calculated for various cut-off levels of placental thickness for different gestational by receiver operating characteristic (ROC) curve.

Results

Of 216 women studied, 30 had a fetus with homozygous α-thalassemia-1 All of these 30 pregnancies developed subtle ultrasonographic signs of fetal anemia (fetal cardiomegaly, pericardial effusion and placentomegaly) during the study period from 16-22 weeks gestation. A few of these cases which observed further into 22-28 weeks' gestation developed signs of hydropic changes (more than two of the following; subcutaneous edema or generalized anasarca, pleural effusion, pericardial effusion and ascites). Hb Bart's diseases were confirmed by cordocentesis and hemoglobin study. These pregnancies were counseled and terminated. No maternal complication was found in these positive cases. The remaining negative cases, 186 women, all

gave birth to normal babies confirmed after birth. There was no Hb Barts' Hydrops Fetalis born in this screened-negative group.

To evaluate the placental sonogram, the subjects were divided into 3 subgroups which were 16-18, 18-20 and 20-22 weeks gestation.

For women in 16-18 weeks subgroup, there were 10 positive cases and 63 negative cases. The sensitivity and specificity in detecting affected pregnancies at cut-off 3.22 cm placental thickness by ROC curve were 0.90 (0.89-1) and specificity 0.96 (0.93-0.99). (Fig.1.)

For pregnant women in the 18-20 weeks subgroup, there were 10 positive cases and 98 negative cases. The sensitivity and specificity in detecting affected pregnancies at cut-off 3.56 cm. placental thickness by ROC curve were 0.90 (0.59-0.98) and 0.96 (0.70-0.98) respectively. (Fig.2.)

For women in the 20-22 week subgroup, there were 10 positive cases and 35 negative cases. The sensitivity and specificity in detecting affected pregnancies at cut-off of 3.85 cm. placental thickness by ROC curve were 0.90 (0.59-0.98) and 0.95 (0.70-0.98) respectively. (Fig.3.)

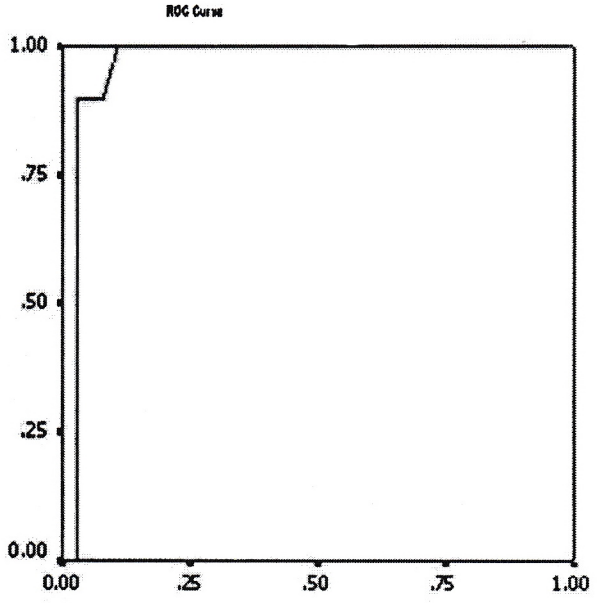

Fig.1. ROC Curve For 16 - 18 weeks Subgroups

At cut-off 3.22 cm

Sensitivity	0.90 (95%CI 0.59-0.98)
Specificity	0.93 (95%CI 0.70-0.98)
Positive Predictive Value	0.90 (95%CI 0.59-0.98)
Negative Predictive Value	0.93 (95%CI 0.70-0.98)

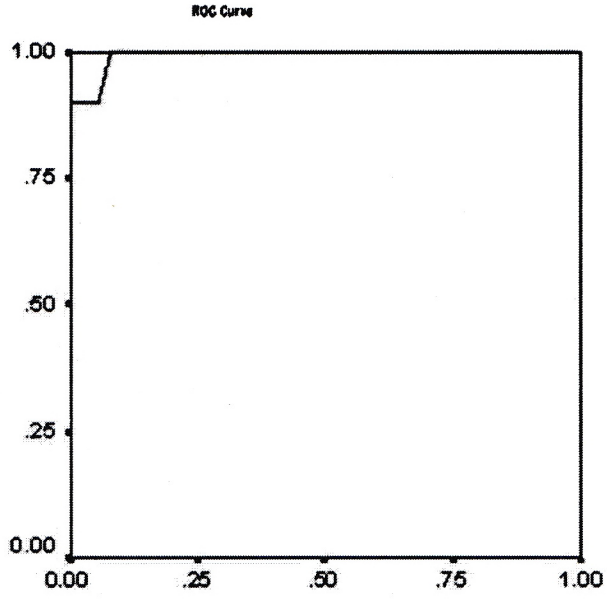

Fig.2. ROC Curve For 18 - 20 weeks Subgroups

At cut-off 3.56 cm

Sensitivity	0.90 (95%CI 0.59-0.98)
Specificity	0.96 (95%CI 0.89-0.98)
Positive Predictive Value	0.75 (95%CI 0.46-0.91)
Negative Predictive Value	0.98 (95%CI 0.92-0.99)

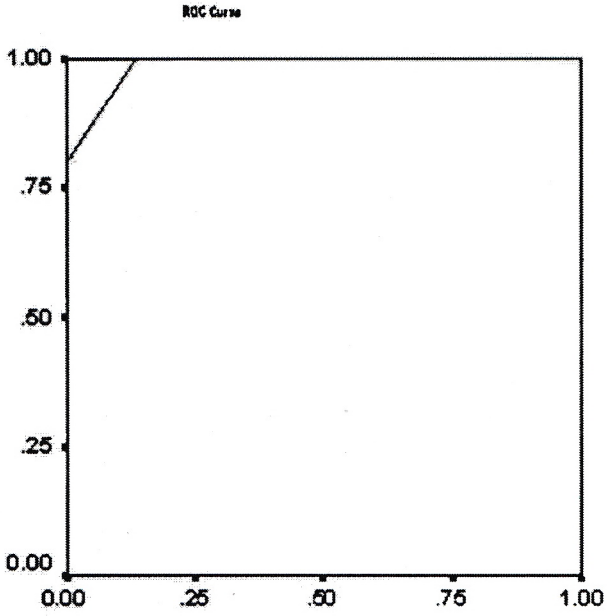

Fig.3. ROC Curve For 20 - 22 weeks Subgroups

At cut-off 3.85 cm

Sensitivity	0.90 (95%CI 0.59-0.98)
Specificity	0.93 (95%CI 0.70-0.98)
Positive Predictive Value	0.90 (95%CI 0.59-0.98)
Negative Predictive Value	0.93 (95%CI 0.70-0.98)

Discussion

Most of the prenatal diagnosis of homozygous α-thalassemia-1 fetuses were made by DNA analysis or by direct hemoglobin electrophoresis after fetal blood sampling. Fetuses affected by homozygous α-thalassemia-1 also develop severe anemia in early fetal life. This lead to hydropic changes including an increase in placental thickness and cardiomegaly. Ghosh A et al have demonstrated an abnormal increase in placental volume preceding cardiomegaly in pregnancies associated with homozygous α-thalassemia-1 and they could identify all affected pregnancies by 18 weeks' gestation.[13] In the late second trimester or third trimester, Hb Bart's hydropic fetuses typically present with ascites, hepatosplenomegaly, oilgohydramnios, and bulky placenta[15-16] which can be easily detected by ultrasound.

Because of the high cost or unavailability of DNA analysis and the risk of fetal loss associated with the invasiveness of cordocentesis, we developed a model of a limited budget prenatal diagnosis of α-thalassemia-1 disease without DNA diagnosis method and selectively use of cordocentesis of Hb Bart's disease confirmation in positive caese. These methods included prospectively universal screening of couple at risk of α-thalassemia-1 trait (identified by low MCV/MCH and normal HbA2 level in both partners) and serial ultrasound from 16-28 weeks gestation and follow up after birth. The prevalence of Hb Bart's disease in pregnancies at risk was only 13.8% in our study, in stead of 25% theoretically. This could be due to the fact that in our screening we assumed that all at-risk patients (low MCV/MCH and normal Hb A2 level) were carriers without confirmation by DNA analysis. Therefore some false positive carriers might be included. Even though it requires more laborious effort in ultrasound screening all suspected at-risk-cases, this method in affordable and non-invasive and it is proved to be efficient enough in detecting early Hb Bart's disease without missing any abnormal cases. However the importance of follow up examination is essential. Since mid trimester ultrasound for routine screening becomes more commonly accepted, it may play an important role in the early screening of Hb Bart's Hydorps fetalis.

Tongsong et al. also demonstrated that measured placental thickness at 18-21 weeks before cordocentesis, at cut-off value 30 mm. gave a sensitivity of 88.52%, specificity of 90.18%, positive predictive value of 78.48% and negative predictive value of 87%.[17] Ko et al. investigated the placental sonogram evaluated against BPD in the early second trimester comparing the affected fetuses already diagnosed by DNA or hemoglobin analysis. They found that the placental thickness generally increased in parallel with the advancement of gestational age and 90% of the placental thickness in affected group was above the mean plus two standard deviations of the control group.[9]

Unlike previous reports, our study measured placental thickness with other signs of fetal sonographic markers propectively and to avoid performing invasive procedures in all at risk cases, we followed these fetuses serially by ultrasound and selectively performed fetal blood sampling (in 18-22 weeks' gestation) in only sonographically abnormal cases. All positive cases were confirmed to be Hb Barts' disease. Comparing with previous reports, our program was safer without

unnecessary fetal loss to unaffected fetuses from invasive procedures.

Due to the fact that the thickness of placenta would be increased in advancing gestational age, in our report, placental thickness values were grouped in 2 weeks period from 16-22 weeks' gestation to provide more accurate values. We have demonstrated that in majority of cases, significant placentomegaly can be detected from 16th week of gestation. On the basis of the ROC curve using those cut-off values of placental thickness measurement has very high accuracy in predicting homozygous alpha-thalassemic fetuses. The sensitivity and specificity in detecting affected pregnancies, at 16-18 week's gestation at cut-off 3.22 cm placental thickness by ROC curve were 95% (0.89-1), specificity 96% (0.93-0.99), positive predictive value 90% and negative predictive value 93%. At 18-20 weeks, at cut-off 3.56 cm. the figures were 90% (0.59-0.98) and 95% (0.70-0.98), positive predictive value 75% and negative predictive value 98%, respectively. At 20-22 week's gestation at cut-off 3.85 cm., the sensitivity, specificity, positive predictive value and negative predictive value were 90% (0.59-0.98), 95% (0.70-0.98), 90% and 93% respectively. Therefore measurement of placental thickness at 16-22 weeks' gestation can differentiate normal from affected pregnancy in suspected at-risk women and should be considered to reduce or obviate the numbers of the invasive procedure needed for diagnosis of Hb Bart's disease.

To avoid error of placental thickness values, special attention should be paid to avoid including the focal myometrium contraction, the fundal or lateral placenta or a tangential section in their measurement, otherwise the placental thickness will be significantly increased.

An abnormal placental thickness is highly suggestive but not absolutely diagnostic of an affected pregnancy and for earlier gestation, some of the abnormal pregnancies could not be detected. Other signs of early fetal hydropic changes such as cardiomegaly and or pericardial effusion should be combined to increase the effectiveness of screening. Invasive diagnostic evaluation of fetal hemoglobin is necessary to confirm diagnosis before termination of pregnancies. Since the main objective of prenatal diagnosis of homozygous α- thalassaemia-1 is reduction of maternal morbidity rather than prevention of birth of affected infants, serial measurement of placental thickness may be an alternative cost-effective method for exclusion of the disorder, particularly in areas where resources for prenatal diagnosis are limited.

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