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Ultrasonographic assessment of pregnancy at risk of homozygous α-thalassaemia-1 in the first trimester

Key Messages

1. Homozygous α-thalassaemia-1 can be predicted reliably using ultrasound measurement of the foetal cardiothoracic ratio at 12 to 13 weeks' gestation, but not at 10 to 11 weeks' gestation.
2. When an optimal foetal cardiothoracic ratio cannot be obtained with an abdominal ultrasound examination at 12 to 13 weeks' gestation, a vaginal ultrasound examination is useful.
3. The use of ultrasonography to rule out homozygous α-thalassaemia-1 can reduce the number of invasive procedures and hence the number of unnecessary foetal losses and laboratory costs for deoxyribonucleic acid studies, without causing diagnostic delays in affected pregnancies.

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Introduction

Thalassaemias are the most common genetic diseases in the world.[1] In Hong Kong, 4.3% of the pregnant population are α-thalassaemia carriers.[2] Couples with α-thalassaemia-1 face a 25% risk of having a foetus with homozygous α-thalassaemia-1. The disease is not yet amenable to treatment. The affected foetuses are either stillborn or die soon after birth. Obstetric complications in women carrying an affected foetus are common. Prenatal diagnosis in the early gestational period allows pregnancy termination. This is conventionally achieved by deoxyribonucleic acid (DNA) analysis of chorionic villi or amniocytes obtained from chorionic villus sampling (CVS) or amniocentesis performed on all at-risk pregnancies. These procedures carry a small but not negligible risk to the foetus.[3] There is a need to develop safer means of prenatal diagnosis.

Affected foetuses have severe anaemia and develop hydropic changes, ie subcutaneous oedema, pleural effusion, pericardial effusion, ascites, etc, that can be detected by prenatal ultrasonography. These hydropic changes are more evident in the third trimester of pregnancy, but can be detected in one third of affected pregnancies at 17 to 18 weeks of gestation.[4] Foetuses at risk of homozygous α-thalassaemia-1 may be examined by serial ultrasound to detect hydropic changes, followed by cordocentesis and haemoglobin (Hb) study to confirm the diagnosis. However, this prenatal diagnostic approach is not sensitive enough before 20 weeks of gestation, when two thirds of the affected pregnancies would be missed. Other subtle signs of foetal anaemia eg placentomegaly and cardiomegaly, detectable by ultrasound examination at earlier gestations need to be evaluated. In a study of 62 at-risk pregnant women who had abdominal ultrasound examinations at 13 to 14 weeks of gestation to assess the foetal cardiac size, a cardiothoracic ratio cut-off of >0.5 was 65% sensitive and 100% specific for disease.[5] However, a good foetal four-chamber cardiac view was not available for computation of the cardiothoracic ratio in 9.7% of cases. We postulate that visualisation of the foetal heart may be improved by the use of vaginal ultrasonography. This may achieve better prediction of homozygous α-thalassaemia-1.

We aimed to determine (1) whether the foetal cardiac size and the placental thickness are increased in the first trimester of pregnancies affected by homozygous α-thalassaemia-1 and (2) whether vaginal ultrasonography is useful for detecting foetuses affected by homozygous α-thalassaemia-1 in the first trimester.

Methods

This study was conducted from July 1996 to December 1997. Women who had given birth to babies with Hb Bart's hydrops foetalis, and couples who had been identified as α-thalassaemia-1 carriers on pre-marital or prenatal screening were recruited. We have been screening all patients for thalassaemia at Tsan Yuk Hospital (TYH)'s antenatal clinics since 1995. Haematological investigations for suspected thalassaemia were performed at the Prenatal Diagnostic and Counselling Laboratory at TYH. All DNA analyses were performed at the DNA Diagnosis Laboratory of the Department of Medicine, The University of Hong Kong. Iron studies were performed at the Biochemistry Laboratory of Queen Mary Hospital.

Maternal blood samples collected at the antenatal booking clinics were sent to the Prenatal Diagnostic Laboratory at TYH where Hb and mean corpuscular volume (MCV) were measured. If MCV was found to be low (<80 fL) in a woman, her partner would be called for MCV testing. If both couples had a low MCV result, we would send the couple's blood for Hb electrophoresis followed by quantitative measurement of Hb A2 using micro-column chromatography, incubation of red blood cells with brilliant cresyl blue for the detection of Hb H inclusion bodies, and iron studies (including iron, percentage of saturation, and ferritin levels). The presence of Hb H inclusion bodies was almost diagnostic of α-thalassaemia-1. Couples with a low MCV result, normal iron status, and normal Hb pattern would be managed as suspected α-thalassaemia trait. DNA analysis would be performed to confirm suspected cases.

Alpha-thalassaemia couples were offered the options of undergoing CVS at 10 to 12 weeks, or amniocentesis at 18 weeks, or having serial ultrasound examinations at 12 to 13 weeks, 16 to 18 weeks, and 30 weeks to detect signs of foetal anaemia (cardiomegaly, placentomegaly, or hydropic changes), followed by cordocentesis and Hb study only in those cases with abnormal findings. All Hb studies of foetal blood samples were performed at the TYH Prenatal Diagnostic Laboratory. We have shown previously that all affected pregnancies can be detected by ultrasound examination at 18 weeks of gestation[5] and cordocentesis is feasible as early as the 12th week of gestation.[6] Between January 1996 and March 1999, 200 women were seen before 14 weeks of gestation and they were recruited for this study after obtaining an informed consent. All subjects had an ultrasound examination at 12 to 13 weeks of gestation to assess its use in the prediction of homozygous α-thalassaemia-1. Initially, we also performed ultrasound examinations at 10 to 11 weeks of gestation in 86 women who presented before 11 weeks.

An abdominal ultrasound examination (Acuson 128XP10, 5 or 7 mHz curvilinear transducer) was always performed first. If the image was not satisfactory, vaginal scanning (Acuson 128XP10, 5 or 7 mHz vector transducer) was performed. The foetal crown rump length was measured to ascertain the gestational age. The foetal cardiac four-chamber view was obtained and the transverse cardiac diameter was taken at the level of the atrio-ventricular valves between the epicardial surfaces during ventricular diastole. The transverse thoracic diameter was measured by placing the pointers just outside the skin. The value was expressed as a cardiothoracic ratio using the transverse cardiac diameter against the transverse thoracic diameter. All measurements were taken before the prenatal diagnostic invasive test results were available. At least two foetal cardiac images were taken and the measurements on the best plane of foetal cardiac four-chamber view were recorded. The lower edge of the ultrasound monitor was covered with dark paper so that the examiner was blinded to the readings while measuring the two diameters. The reproducibility of the cardiothoracic ratio was assessed in a subgroup of 50 subjects by comparing two measurements on the best foetal plane. The intra-observer repeatability was less than 0.02 in 95% of cases. Ultrasound findings of six of the 49 affected foetuses in this study have been reported previously as a pilot study.[6]

Diagnosis and data processing/analysis

The diagnosis of homozygous α-thalassaemia-1 was confirmed by DNA study (absence of α genes) or by Hb study (anaemia and the presence of more than 80% of Hb Bart's). Pregnancy outcomes were further ascertained by following up the babies at birth, including those who had not been subjected to prenatal invasive testing. Cord blood haematological confirmation was performed in the affected and the unaffected cases after birth at the TYH Prenatal Diagnostic Laboratory. All cord blood results and outcomes were traced by nurse specialists.

The use of ultrasound measurements for prediction of homozygous α-thalassaemia-1 was assessed by sensitivity, specificity, and predictive values. The differences between measurements in unaffected and affected pregnancies were analysed by the two-tailed Student's *t*-test using the Statistical Package for Social Sciences (Windows version 6.0; SPSS Inc, Chicago [IL], US).

Results

We abandoned scanning at 10 to 11 weeks in the later part of the study because a cardiothoracic ratio could not be reliably obtained in the majority of cases, and the examination was not predictive of disease. We did not perform vaginal ultrasonography in all women because in most, abdominal examination at 12 to 13 weeks of gestation gave an optimal image, while some women did not agree to a vaginal examination. Of the 200 subjects studied, 43 (22%) chose to have CVS, 26 (13%) chose to have amniocentesis, and 131 (66%) chose to have serial ultrasound examination, followed, if positive, by cordocentesis.

Vaginal ultrasonography

An optimal cardiothoracic ratio could be obtained in only 40 (47%) of 86 women who received an ultrasound examination at 10 to 11 weeks of gestation. Vaginal ultrasonography was performed in 54 women at 10 to 11 weeks of gestation, producing optimal images in 30 (56%) of them. When ultrasound examinations were performed in all 200 subjects after 11 weeks, an optimal cardiothoracic ratio was obtained in 135 subjects at 12 weeks, and in 65 subjects at 13 weeks. Of 69 subjects who received a vaginal examination at 12 to 13 weeks, a satisfactory image was obtained in all of them.

Outcomes

A total of 151 foetuses were unaffected by homozygous α-thalassaemia-1. Of these, 97 belonged to the group of women who chose to have serial ultrasound examination. All findings were normal so cordocentesis was not performed. Forty-nine foetuses were affected by homozygous α-

Table 1. Gestation at diagnosis of the 49 pregnancies affected by homozygous α-thalassaemia-1

Gestation at diagnosis	Initial mode of prenatal diagnosis		
	Chorionic villus sampling (n=43)	Amniocentesis (n=26)	Ultrasound* (n=131)
12 weeks	6	2†	17†
13 weeks	4	2†	15†
14 weeks	0	0	1‡
15 weeks	1	1†	0

* Serial ultrasound examinations followed by cordocentesis on those with abnormal findings
† Diagnosis was made by cordocentesis and haemoglobin study
‡ Diagnosis was made by chorionic villus sampling and deoxyribonucleic acid analysis

Table 2. The foetal cardiothoracic ratio, transverse cardiac diameter, thoracic diameter and placental thickness at 10 to 11 weeks and 12 to 13 weeks in the pregnancies unaffected and affected by homozygous α-thalassaemia-1

Ultrasonographic measurement	Unaffected Mean (SD)	Affected Mean (SD)	Difference Mean (95% CI)	P value (*t* test)
At 10-11 weeks	n=31	n=9		
Cardiothoracic ratio	0.47 (0.40)	0.48 (0.06)	0.02 (-0.02 to 0.05)	0.280
Transverse cardiac diameter (mm)	6.2 (0.8)	6.7 (7.7)	0.5 (-0.1 to 1.1)	0.086
Thoracic diameter (mm)	13.4 (1.7)	13.4 (2.2)	0.0 (-1.3 to 1.4)	0.955
Placental thickness (mm)	11.9 (3.3) [n=50]	12.4 (4.3) [n=22]	0.5 (-1.4 to 2.3)	0.612
At 12-13 weeks	n=151	n=49		
Cardiothoracic ratio	0.45 (0.02)	0.54 (0.03)	0.09 (0.08 to 1.00)	<0.001
Transverse cardiac diameter (mm)	8.0 (1.1)	9.0 (1.0)	1.0 (0.6 to 1.3)	<0.001
Thoracic diameter (mm)	18.6 (1.8)	16.7 (2.0)	-0.9 (-1.5 to -0.3)	0.004
Placental thickness (mm)	16.4 (4.6)	21.5 (4.7)	5.1 (3.6 to 6.6)	<0.001

Table 3. The sensitivities, specificities and predictive values (%) with each cut-off level of the foetal cardiothoracic ratio and placental thickness at 12 to 13 weeks in the prediction of the pregnancies affected by homozygous α-thalassaemia-1

Cut-off level	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Cardiothoracic ratio				
≥0.48	100.0	84.8	68.1	100.0
≥0.50	100.0	99.3	98.0	100.0
≥0.53	69.4	100.0	100.0	91.0
Placental thickness (mm)				
≥15	91.8	31.8	30.4	92.3
≥18	81.6	67.5	44.9	91.9
≥20	73.5	86.1	63.2	90.9
≥22	53.1	94.0	74.3	86.1
≥24	28.6	98.0	82.4	80.9

thalassaemia-1. The diagnosis was made by CVS and DNA study in 12 cases, and by cordocentesis and Hb study in 37 cases. Table 1 shows the gestational age at diagnosis of the affected foetuses according to the women's initial choice of mode of prenatal diagnosis. Five belonged to the group who originally chose to have amniocentesis, but were advised to undergo cordocentesis because ultrasound scans showed enlarged foetal hearts well before the scheduled amniocentesis.

Ultrasonographic findings

Table 2 shows the ultrasonographic foetal cardiothoracic ratio, transverse cardiac diameter, thoracic diameter, and placental thickness measurements at 10 to 11 weeks and 12 to 13 weeks of gestation in unaffected and affected pregnancies. There were no significant differences in all four measurements at 10 to 11 weeks of gestation, however, at 12 to 13 weeks, the cardiothoracic ratio of the affected foetuses was significantly greater than the unaffected ones. The transverse cardiac diameter was significantly greater while the thoracic diameter was significantly smaller in the affected foetuses. The placental thickness in the affected pregnancies was significantly greater than the unaffected ones. Table 3 shows the sensitivities, specificities and predictive values for each cut-off level of the foetal cardiothoracic ratio and placental thickness for prediction of affected pregnancies. With a cardiothoracic ratio cut-off of ≥0.5, all 49 affected foetuses were identified. Only one unaffected foetus had a cardiothoracic ratio cut-off of >0.5 and was subjected to an invasive test. The sensitivity, specificity, positive and negative predictive values were 100%, 99.3%, 98% and 100% respectively.

Discussion

Foetuses affected by homozygous α-thalassaemia-1 have deficient α-globin synthesis. They are anaemic and the Hb concentration is between 60-80 g/L.[1] Their Hb consists mainly of Hb Bart's (γ_4) and Hb Portland ($\zeta_2\gamma_2$) instead of Hb F($\alpha_2\gamma_2$).[1] As Hb F becomes the major foetal Hb from 8 weeks of gestation, we can assume that the affected foetuses are anaemic in the first trimester of pregnancy. Hb Bart's has a very high oxygen affinity and has no Bohr effect. The affected foetuses are more acidotic, hypoxic and hypercarbic than normal ones. They compensate for

the hypoxia and anaemia by increasing cardiac output. An ultrasound Doppler blood flow study of 32 foetuses with Hb Bart's disease between 18 to 26 weeks of gestation, showed that their increased cardiac output is accomplished mainly by dilating the cardiac chambers to twice the normal size.[7] We have shown recently that cardiomegaly can be observed by ultrasound examination in 65% of affected foetuses at 13 to 14 weeks of gestation.[5] However, only abdominal scanning was performed in that study, which might account for its low sensitivity. Our data show that vaginal scanning at 12 to 13 weeks of gestation was useful in all 69 women (34.5% of the total) in whom satisfactory images could not be obtained with abdominal scanning. We can also reliably predict homozygous α-thalassaemia-1 by measuring the foetal cardiothoracic ratio at 12 to 13 weeks of gestation. In our experience, the abdominal and vaginal scanning approaches are often complementary to each other. Abdominal scanning allows free manipulation to get the appropriate foetal plane, but the clarity is occasionally compromised by thick maternal abdominal walls or foetal posture. In those cases, vaginal scanning may be useful. The role of vaginal scanning at 10 to 11 weeks is limited because the foetal cardiothoracic ratio could not be reliably obtained in 44.4% (24/54) of subjects, and the examination findings were not predictive of the disease.

In the group of 131 women who chose to have serial ultrasound examinations followed by cordocentesis if the ultrasound findings were abnormal, 33 foetuses were affected by homozygous α-thalassaemia-1. In all except one of them, the diagnoses were made at 12 to 13 weeks of gestation (Table 1). This was possible because the ultrasound feature was distinctive and confirmation by Hb study of foetal blood obtained from cordocentesis took only 1 to 2 days. Confirmation of the diagnosis at 12 to 13 weeks of gestation is beneficial because pregnancy termination at this stage is safer and psychologically more acceptable than that done at a more advanced gestation. On the other hand, cordocentesis should not be performed lightly because it is associated with a 5 to 9% risk of foetal loss.[6] We think that one should acquire experience in foetal echocardiography, especially the measurement of the cardiothoracic ratio before embarking on this diagnostic approach. In experienced hands, we have shown that only one of the 149 unaffected pregnancies was subjected to an invasive test unnecessarily. This should prevent procedure-related foetal loss. At the same time, there was no delay in confirming the diagnosis in the affected pregnancies (Table 1). This diagnostic approach is especially valuable in parts of South-East Asia where the disease is prevalent but DNA study is not readily available. In centres where CVS and DNA study are readily available, this approach may limit invasive procedures in those identified as at high risk by ultrasound examination. Since DNA study is expensive, the medical saving is likely to be substantial. An alternative to cordocentesis at 12 to 13 weeks is CVS and polymerase chain reaction analysis, which can give a rapid result within 2 days. The risk of foetal loss following CVS (about 1%) is much less than that following cordocentesis. Although the placental thickness in the affected pregnancies was significantly larger than that in the unaffected ones, we cannot find a cut-off level of placental thickness that is associated with a satisfactory sensitivity and specificity. On the other hand, a much better result is obtained when a cut-off level of 0.5 is used for the foetal cardiothoracic ratio.

This study represents preliminary work in the prenatal prediction of homozygous α-thalassaemia-1 early in the second trimester. While satisfactory and useful foetal cardiothoracic ratio measurements at 12 to 13 weeks of gestation can be produced by one investigator, similar results may not be reproduced by others. Further studies should assess the learning curve and inter-observer variability for measurement of the foetal cardiothoracic ratio. We also believe that an ultrasound machine able to produce a high image resolution is needed to obtain optimal foetal cardiothoracic ratios at 12 to 13 weeks of gestation. A satisfactory cardiothoracic ratio measurement may not be obtained in a machine with a suboptimal resolution. Lastly, the subjects in our study were women who had given birth to babies with Hb Bart's hydrops foetalis, or couples who were identified to as α-thalassaemia-1 carriers on screening. Our study results may not be applicable to larger populations suspected to have α-thalassaemia trait because of a low MCV value (<80 fL) only.

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