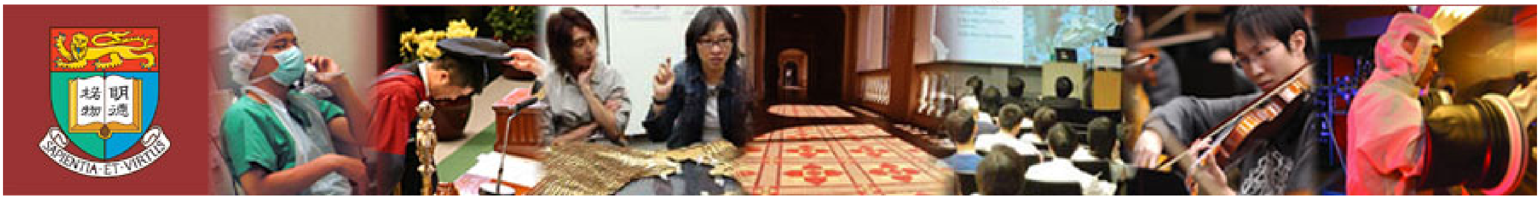

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Author(s)	Leung, KY; Cheong, KB; Tang, MHY; Chan, V
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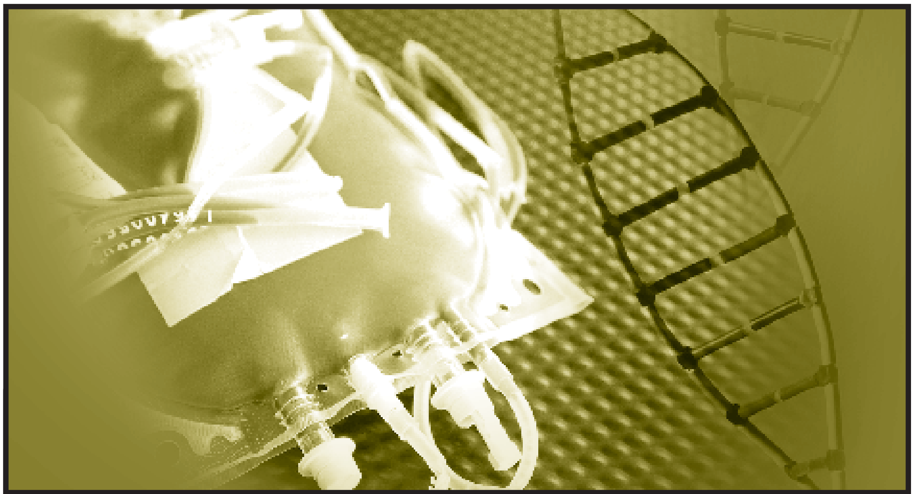
Prenatal Diagnosis of Thalassaemia

KY Leung, MBBS, FRCOG, FHKAM(O&G); **KB Cheong,** MBBS, MMedSc; **MHY Tang,** MBBS, FRCOG, FHKAM (O&G);
Vivian Chan, MSc, PhD (Lond), DIC, FACB, FRCPath, HonFRCP (Lond), HonFHKCP



The two major types of thalassaemias are transfusion-dependent β-thalassaemia major and homozygous α^0-thalassaemia

INTRODUCTION

Thalassaemias are a major public health problem in many parts of the world.[1] They are a family of inherited disorders of haemoglobin synthesis characterized by a reduced output of one or other of the globin chains of adult haemoglobin. The two main disorders are transfusion-dependent β-thalassaemia major and homozygous α^0-thalassaemia. Although fetuses affected by homozygous α^0-thalassaemia usually die in-utero or in the early neonatal period, prenatal diagnosis is still justified because of maternal morbidities, including pre-eclampsia, haemorrhage, the adverse psychological impact of carrying a hydrops fetalis to term only to result in a still-birth, and, occasionally, death.[2] Homozygous haemoglobin E (HbE) is associated with mild anaemia only, but coinheritance of β-thalassaemia may result in a transfusion-dependent state. The coinheritance of the sickle-cell gene and β-thalassaemia produces a disease that is similar to sickle-cell anaemia. Thalassaemia is an autosomal recessive disorder, and if both the mother and father carry the same thalassaemia trait, their offspring will have a 1 in 4 chance of having thalassaemia major. The prevalence of different types of thalassaemia trait is up to 16% in Cypriot populations; 3% to 14% in Chinese and Thai populations; 3% to 8% in Indian, Pakistani and Bangladedeshi populations; 0.9% in black Caribbean and African populations; and 0.1% in northern Europeans.[3-5]

Prenatal screening has been introduced worldwide to prevent severe thalassaemias,[6-8] and has shown to be cost-effective in many places including the United Kingdom, Quebec, Israel and Hong Kong.[9-13] The introduction of a preventive programme has resulted in a marked decline in the number of newborns with β-thalassaemia major.[14] The choice of screening strategy used depends on the ethnic origin of the local population, as the latter has its own characteristic spectrum of haemoglobin variants and thalassaemia mutations. Conventionally, prenatal diagnosis is performed using amniocentesis or chorionic villus sampling (CVS) followed by DNA analysis. Compared to the original gene mapping method, recently-developed polymerase chain reaction (PCR) methods and arrays are simpler, faster and less expensive, but PCR methods are associated with specific diagnostic pitfalls.[15] A noninvasive approach using serial ultrasound examinations has been shown to effectively reduce the need for an invasive test in pregnancies unaffected by homozygous α^0-thalassaemia. Other methods, including assessment of maternal serum markers, maternal plasma fetal erythrocytes, maternal plasma fetal DNA, and three-dimensional ultrasound examination, have been investigated. The aim of the present review article is to provide an update on the clinical aspects of prenatal screening and diagnosis of severe thalassaemias.

ANTENATAL SCREENING

Universal antenatal screening for thalassaemia has been advocated for populations with a high prevalence of asymptomatic thalassaemia trait. With population migration, thalassaemias are no longer regionally specific. Selective prenatal screening tests have been offered to at-risk couples because of their ethnic origin. Web-based population and genetic information on thalassaemia mutations and variants is available for easy reference.[16]

There is no universal screening policy which can suit all countries because of the heterogeneity of thalassaemias and the association with haemoglobin variants in different ethnic groups. For populations with thalassaemias as the major health problem, measurement of maternal mean corpuscular volume (MCV) or mean corpuscular haemoglobin (MCH) has been adopted as a screening parameter.[17] Both MCV and MCH are useful as screening for α- and β-thalassaemia traits. The choice depends on the familiarity of the local laboratory with the measurement of each parameter, and whether storage of blood before testing for MCV/MCH is required. MCH has the theoretical advantage over MCV because it is more stable over time. For populations with a high prevalence of sickle cell and β-thalassaemia traits, haemoglobin electrophoresis as well as MCV/MCH should be performed for screening because the latter is not useful for detecting sickle cell trait.

Different MCV cutoffs between 76 fL and 82 fL have been used. By genotyping archived samples from schoolchildren with an MCV up to 85 fL, it has been shown that a MCV cutoff of 80 fL or MCH cutoff of 27 pg could detect all Southeast Asia (SEA) deletion carriers and β-thalassaemia carriers.[18] With MCH, 27 pg seems to be the most acceptable cutoff.

Prenatal screening for thalassaemia has resulted in a marked decline in the number of affected newborns

If maternal MCV or MCH is found to be low, checking the partner's blood will be required. If the partner's MCV or MCH is also low, α^0-thalassaemia trait, β-thalassaemia trait or iron deficiency need to be excluded in both partners.[19] Laboratory tests to look for the presence of HbH inclusion bodies and elevation in HbA2 should be performed, as should an iron study. The presence of HbH-inclusive inclusion bodies and elevation of HbA2 (>3.5%) are diagnostic of α^0-thalassaemia trait and β-thalassaemia, respectively. Although normal A2 β-thalassaemia exists, it is extremely rare. If HbA2 level is normal, HbH inclusion bodies are absent, and iron deficiency is detected, women should be treated with iron therapy first, followed by repeat MCV or MCH 4 weeks later, before further investigations on their thalassaemia status.[19] An increase in their Hb by 1 g/dL and an increase in MCV after iron therapy for 4 weeks can be expected. In some ethnic groups such as Cypriots and Sardinians, the possibility of δβ-thalassaemia should be suspected in women with low MCV/MCH who have normal HbA2. δβ-thalassaemia and the deletion types of hereditary persistence of fetal haemoglobin (HPFH) are characterized by an elevated level of HbF (5%-30%) in heterozygotes.[20]

Couples with confirmed or suspected thalassaemia traits are preferably counselled by specialists in maternal-fetal medicine or specialists with an understanding of prenatal diagnosis of thalassaemias. DNA analysis will be performed to confirm suspected cases. In the presence of a low MCV and a normal iron status, absence of HbH inclusion bodies does not necessarily exclude α-thalassaemia, and DNA analysis is required to detect α-thalassaemia trait. Couples carrying α^0-thalassaemia or β-thalassaemia minor have a 1 in 4 chance of having infants with homozygous α^0-thalassaemia or β-thalassaemia major, respectively. For couples with discordant thalassaemia traits, their fetus will not be at risk of serious thalassaemia unless one parent who carries β-thalassaemia trait also has coinheritance of α^0-thalassaemia. In regions with a high prevalence of both α- and β-thalassaemia, up to 7% of β-thalassaemia carriers are compound α- and β-thalassaemia heterozygotes,[21] and ζ-mapping should thus be performed to exclude α-thalassaemia. If one parent has β-thalassaemia trait and the other has E-thalassaemia trait, their fetus will have a 1 in 4 risk of being compound heterozygous for β- and E-thalassaemia traits, which can be transfusion-dependent in the more severe phenotype.

Early screening allows time for workup and counselling for couples who are at risk. In case termination of pregnancy needs to be considered, timely diagnosis of thalassaemia major in the fetus is desirable. For populations with a high prevalence of α-thalassaemia carriers, antenatal screening should be offered to all pregnant women regardless of gestational age, in view of maternal risks including pre-eclampsia and postpartum haemorrhage.

Education of healthcare professionals,[22] information for couples and informed consent are required before screening. Information for couples may be provided through pamphlets, videos, websites or explanation in person. At-risk couples must be counselled on the maternal risks associated with affected pregnancies, together with the prognosis, available support and treatment for affected infants. They also need to be informed of the available invasive and noninvasive prenatal tests, risk of sampling, limitation and accuracy of prenatal tests, screening workflow, and the need for confirmation testing at delivery.

Prenatal screening for thalassaemia usually includes the measurement of maternal MCV/MCH with or without haemoglobin electrophoresis

Screening Pitfalls

If Hb electrophoresis is not performed as part of the initial screening, there is a possibility of missing some sickle cell carriers or HbE carriers whose MCVs are above 80 fL.[23] As a result, sickle cell disease (HbS/β-thalassaemia) or β-thalassaemia syndrome (β-thalassaemia/HbE) will be missed if the spouse carries β-thalassaemia trait. Whether Hb electrophoresis should be performed as part of the routine screening depends on the prevalence of HbE and sickle cell trait in a local population and on resources. In Hong Kong, Hb electrophoresis is not performed routinely because HbS is not found in the Chinese population.[13]

There is no universal screening policy which can suit all countries because of the heterogeneity of thalassaemias and the association with haemoglobin variants in different ethnic groups.

When iron deficiency is accompanied by a normal HbA2 level and an absence of HbH inclusion bodies, the approach of giving iron therapy for 4 weeks and then repeating MCV measurement before performing further investigations on thalassaemia status may lead to a delay in the prenatal diagnosis of both α- and β-thalassaemia. There is a possibility of concomitant thalassaemia trait and iron deficiency because in the presence of iron deficiency, HbA2 production may be depressed,[24] and the brilliant cresyl blue preparation used for the detection of HbH inclusion bodies may be affected. Either DNA analysis to exclude the possibility of α^0-thalassaemia or serial ultrasound examinations to look for features of homozygous α^0-thalassaemia, as described below, should be performed.

The above antenatal thalassaemia screening programmes are not aimed at couples who are at risk of having children with HbH disease. In α+-thalassaemia carriers (with single α-globin gene deletion or nondeletion α-globin gene mutation such as Hb Constant Spring), MCV can lie between 80 fL and 85 fL, and Hb electrophoresis gives normal results. The couple will have a chance of having a child with HbH disease if the spouse carries α^0-thalassaemia. However, prenatal diagnosis of HbH disease is usually not indicated because the affected individual is not transfusion-dependent and can enjoy a normal life. Rare cases of HbH hydrops fetalis have been reported.[25,26] It is therefore necessary to determine the type of nondeletion α+-thalassaemia mutation of the parent.

In the presence of a low MCV and a normal iron status, absence of HbH inclusion bodies does not necessarily exclude α-thalassaemia

For couples with α^0-thalassaemia, serial ultrasound examinations by experienced personnel can reduce the need for invasive testing.

PRENATAL DIAGNOSIS

When at-risk couples are identified, counselling and prenatal testing should be carried out by personnel and laboratories with experience in prenatal diagnosis.[17]

Prenatal Diagnosis of β-thalassaemia Major

As the type of DNA mutations present in each individual varies, it is important to know the ethnic origin of each partner and allow some time for the laboratory to identify the mutation. A thalassaemia (thal) array has been designed to speed up this process.[27] After identification of the parental mutations, the fetal diagnosis is often accomplished by an invasive procedure (chorionic villus sampling [CVS] or amniocentesis), followed by DNA analysis. Occasionally, the mutations cannot be identified, and one may need to resort to cordocentesis and globin chain assay for prenatal diagnosis.[28] However, cordocentesis is associated with a 2% risk of miscarriage.[29]

To avoid an invasive procedure, examination of the circulatory fetal DNA in maternal plasma for mutant paternal allele has been investigated. If paternal mutation is different from maternal mutation and the former is absent from maternal plasma, it will be inferred that the fetus has not inherited the mutant paternal allele, and β-thalassaemia major can be excluded. However, if paternal mutation is present in the maternal plasma, an invasive procedure will still be needed because whether the fetus has inherited the maternal mutation cannot be determined from examination of the maternal plasma.[30,31] However, there are several technical challenges, including failure to isolate or extract fetal DNA from maternal plasma, or PCR allele dropout.[31] The use of advanced techniques such as single allele base extension reaction and Mass ARRAY system may be helpful.[31] Further studies are needed before this noninvasive approach can be used clinically.

Prenatal diagnosis is usually performed by CVS or amniocentesis, followed by DNA analysis

Prenatal Diagnosis of Homozygous α^0-thalassaemia

Homozygous α^0-thalassaemia can be accurately diagnosed by DNA study of cells obtained from CVS or amniocentesis.[32] With the use of quantitative PCR assay, a report can be rapidly available within 1 or 2 days after amniocentesis or CVS.[33] In areas where DNA diagnosis is not easily available, the more invasive technique of cordocentesis, followed by haemoglobin analysis, is an alternative. In affected fetuses, fetal anaemia is found, and haemoglobin electrophoresis shows the presence of Hb Bart's and Hb Portland.

With experienced personnel and a good ultrasound machine, serial ultrasound examinations at 12 to 15, 18 to 20, and 30 weeks' gestation can effectively reduce the need for invasive testing in the majority of unaffected pregnancies.[19,34,35] If there is fetal cardiomegaly (cardiothoracic ratio [CTR] ≥0.50, 0.52, and 0.59 at 12 to 15, 18 and 30 weeks' gestation, respectively), and/or placentomegaly (placental thickness >18 mm at 12 weeks' gestation), CVS or amniocentesis for fetal DNA analysis should be performed for confirmation of α^0-thalassemia major.[19] On the other hand, if ultrasound examinations are normal, an invasive procedure can be omitted. This approach is applicable in singleton as well as twin pregnancies,[36] and can be used to confirm normality in pregnancies conceived after preimplantation genetic diagnosis.[37]

Ultrasound Diagnostic Pitfalls

There are several limitations of the noninvasive approach of using serial ultrasound examinations. Firstly, measurement of placental thickness may be inaccurate if the placenta is adjacent to a focal myometrial contraction, or located in the fundus or lateral uterine wall. In an affected pregnancy, the placenta can be large but its thickness can be normal.[38] Secondly, the predictive values of fetal CTR decrease with gestational age.[19] In advanced gestation, hydropic signs, including ascites and pleural effusion, are more apparent than cardiomegaly in affected pregnancies.[19] Measurement of fetal middle cerebral artery peak systolic velocity may be useful.[20,39] Thirdly, the false-positive rate of this noninvasive approach is about 3% because disorders such as intrauterine growth restriction, congenital heart disease,[19] and HbH disease can present with cardiomegaly and/or placentomegaly. An invasive test is needed to confirm the diagnosis of an affected fetus. Fourthly, this noninvasive approach demands accurate measurement of the fetal CTR. Adequate training and subsequent quality control are essential. When the image quality of the fetal heart at 12 weeks' gestation is suboptimal – even with the use of a transvaginal scan – rescanning in 2 to 3 weeks would be a reasonable option if the mother still prefers ultrasound monitoring to invasive testing.[19] The risk of delaying the diagnosis of an affected pregnancy until the second trimester and the disadvantages of terminating an affected pregnancy in the second trimester should be balanced against the risk (1%) of invasive testing.

Other noninvasive methods have been investigated. If a woman opts for first trimester combined Down's syndrome screening, normal maternal serum-free β human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) at 11 to 14 weeks of gestation is a reassuring sign of normality for fetuses at risk of homozygous α^0-thalassaemia. A high level of free β-hCG, however, is not predictive of affected fetuses.[40] Immunofluorescence staining of fetal erythrocytes in maternal blood is a potentially useful but labour-intensive noninvasive technique in the first trimester.[41] In affected pregnancies, positive staining with fluorescence-labelled monoclonal anti-zeta (but not with anti-alpha) globin antibodies can identify fetal non-nucleated red blood cells. More recently, it has become feasible to provide a potentially rapid noninvasive test using real-time quantitative seminested PCR,[42] or a multiplex quantitative fluorescent PCR (QF-PCR) test.[43] The maternal blood is examined using simultaneous detection of homozygous α^0-thalassaemia by the absence of both microsatellite markers located within breakpoints of the SEA deletion, and exclusion of maternal contamination by the absence of noninherited maternal alleles.[44]

Laboratory Diagnostic Pitfalls

Most of the errors in diagnosis result from maternal contamination, or nonpaternity or technical problems of DNA analysis.[44] If prenatal diagnosis of α^0-thalassaemia is made solely by PCR assay on fetal DNA, errors in diagnosis may occur due to failure of the PCR assay to generate a specific DNA fragment.[45] Maternal contamination can be reduced by using careful invasive techniques, dissecting maternal decidua from the fetal trophoblast, and reducing the number of amplification cycles to 25.[46] If the fetal diagnosis is identical to the maternal genotype, checking maternal contamination by DNA polymorphism analysis to avoid the possibility of misdiagnosis will be required.

CONCLUSIONS

Prenatal screening for thalassaemia usually includes the measurement of MCV/MCH with or without Hb electrophoresis, depending on the ethnic origin of the local population. When at-risk couples are identified, counselling and prenatal testing should be carried out by personnel and laboratories with experience in prenatal diagnosis. Prenatal diagnosis is usually performed by CVS or amniocentesis, followed by DNA analysis. For couples who have α^0-thalassaemia, with experienced personnel and good ultrasound machines, serial ultrasound examination can be offered, reserving invasive diagnosis for those showing ultrasound features of an affected pregnancy.

About the Authors

Dr Leung is Consultant and Dr Cheong is a Ph student at the Department of Obstetrics and Gynaecology, University of Hong Kong, Queen Mary Hospital; Dr Tang is Consultant at the Prenatal Diagnostic and Counselling Department, Tsan Yuk Hospital; Professor Chan is Professor at the Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong SAR, China.
E-mail: leungky1@ha.org.hk

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The Quiz

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Answers are shown at the bottom of this page. We hope you enjoy learning with *JPOG*.

CME Article:

Prenatal Diagnosis of Thalassaemia

Answer True or False to the questions below.

	True	False
1. Measurement of maternal mean corpuscular volume (MCV) or mean corpuscular haemoglobin (MCH) is useful for prenatal screening for thalassaemia.	○	○
2. A normal haemoglobin pattern can exclude the possibility of α-thalassaemia.	○	○
3. If one parent carries β-thalassaemia trait while the other carries α-thalassaemia, their fetus will not be at risk for serious thalassaemia unless the parent who carries β-thalassaemia trait has coinheritance of α-thalassaemia.	○	○
4. If both the father and the mother carry haemoglobin E trait, their fetus will be at risk of severe thalassaemia.	○	○
5. In the presence of iron deficiency, the measurement of haemoglobin A2 production and detection of haemoglobin H inclusion bodies may be affected.	○	○
6. In the prenatal diagnosis of β-thalassaemia major, it is important to know the ethnic origin of each partner.	○	○
7. In the prenatal diagnosis of homozygous α^0-thalassaemia, with the use of quantitative polymerase chain reaction assay, a report can be rapidly available within 1 or 2 days after amniocentesis or chorionic villus sampling.	○	○
8. Serial prenatal ultrasound examinations can effectively reduce the need for invasive testing in the majority of pregnancies unaffected by homozygous α^0-thalassaemia.	○	○
9. Placental thickness is the most useful ultrasonographic parameter in the prediction of pregnancies affected by homozygous α^0-thalassaemia.	○	○
10. In the prenatal diagnosis of thalassaemia, maternal contamination should be suspected if the fetal diagnosis is identical to the maternal genotype.	○	○

Answers

1	2	3	4	5	6	7	8	9	10
T	F	T	F	T	T	T	T	F	T