Seminars in Fetal & Neonatal Medicine (2008) 13, 215–222

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Seminars in FETAL & NEONATAL MEDICINE

Alpha-thalassaemia

W.C. Leung [a,*], K.Y. Leung [b], E.T. Lau [b], M.H.Y. Tang [b], V. Chan [c]

[a] Department of Obstetrics & Gynaecology, Kwong Wah Hospital, 25, Waterloo Road, Kowloon, Hong Kong
[b] Prenatal Diagnostic and Counselling Department, Tsan Yuk Hospital, Hong Kong
[c] Department of Medicine, Queen Mary Hospital, University of Hong Kong, Hong Kong

KEYWORDS
Alpha-thalassaemia;
Haemoglobin (Hb) Bart's;
Pre-implantation genetic diagnosis;
Prenatal diagnosis;
Screening

Summary Alpha-thalassaemia is one of the most common human genetic disorders. Couples in which both partners carry alpha^0-thalassaemia traits have a 25% risk of having a fetus affected by homozygous alpha-thalassaemia or haemoglobin Bart's disease, with severe fetal anaemia *in utero*, hydrops fetalis, stillbirth or early neonatal death, as well as causing various maternal morbidities. This disorder is common in southeast Asia and southern China, and the expanding populations of southeast Asian immigrants in the US, Canada, UK and Europe mean that this disorder is no longer rare in these countries.

Introduction

The thalassaemias (thal) are a group of hereditary anaemias caused by the defective production of one of the globin chains of haemoglobin (Hb). This is the most common single gene disorder in the Mediterranean region and Southeast Asia, affecting 4.8% of the world population.[1]

The Hb molecule is a tetramer consisting of two pairs of globin chains, each containing a haem group, which is responsible for oxygen delivery to the tissues. During fetal development, the fetal Hb predominates ($\alpha 2\ \gamma 2$). In the normal adult, HbA ($\alpha 2\ \beta 2$) constitutes around 95% of circulating Hb. Thus, the most common forms of thal are the α- and β-thals.

The α-globin gene cluster located on chromosome 16 p13.3 comprises the embryonic ζ-globin gene and two α-globin genes $\alpha 2$ and $\alpha 1$, in tandem (in *cis*).[2] The normal complement of α-globin genes is four. Deletions or mutations affecting one or more α-globin genes, resulting in decreased or absent production of the α-globin chain, will give rise to α-thal. The clinical severity of the disease depends on the number of α-globin genes involved. Individuals with the loss of either one (α^+-thal) or two α-globin genes on the same chromosome (in *cis*, α^0-thal), or one on each of the two chromosome 16 (in *trans*, homozygous α^+-thal) are usually asymptomatic. Some might have borderline anaemia, but most will have hypochromic microcytic red blood cells.[3] Those with loss of function of three genes [Hb H ($\beta 4$) disease][4] have a thal intermedia picture, which is characterized by moderate anaemia, marked hypochromic microcytic red cells and splenomegaly.[5] Complete deletion of all four α-globin genes [Hb Bart's ($\gamma 4$) hydrops fetalis][6] causes severe anaemia *in utero*, resulting in death in late gestation (at around 36 weeks onwards) or shortly after birth. Mothers carrying such hydropic fetuses are prone to an increased incidence of pregnancy-induced hypertension (up to 80%), antepartum haemorrhage, malpresentation, prematurity, fetal distress, difficult vaginal delivery, caesarean section, retained placenta and postpartum haemorrhage.[7] Thus, prenatal diagnosis of pregnancies at risk for homozygous α^0-thal and early termination of the

* Corresponding author. Tel.: +852 3517 5056; fax: +852 3517 5269.
E-mail address: leungwc65@hotmail.com (W.C. Leung).

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doi:10.1016/j.siny.2008.02.006

hydrops fetalis will be necessary to avoid maternal morbidities and mortalities as well as the psychological trauma of carrying a hydropic fetus to term only to result in a stillbirth.

This chapter reviews various aspects of alpha-thalassaemia, including its molecular basis, antenatal screening, invasive prenatal diagnosis, non-invasive (ultrasound and maternal blood) prenatal diagnosis and pre-implantation genetic diagnosis.

Antenatal screening

As homozygous α^0-thal (Hb Bart's hydrops fetalis) is an autosomal recessive condition, it is necessary to identify couples at risk. Worldwide, around 4.5% of people carry a haemoglobinopathy gene.[8] As a whole, population screening for thal carriers might not be practical in most areas but selective screening, through the antenatal clinics, might be a more suitable approach. Antenatal screening programmes are established in many places throughout the world[9]; one has been in place in Hong Kong since 1988.[10]

It is well known that the majority of people with α^0-thal (deletions of two α-globin genes in *cis* on the same chromosome) are not anaemic but do have microcytosis, i.e. a low mean corpuscular volume (MCV). Hence, most screening programmes rely on the initial identification of at-risk couples by their microcytosis (MCV $<$ 82 fl), in the absence of iron deficiency.[11,12] Within a local population that comprises virtually 95% ethnic Chinese, a cut-off value for MCV $<$ 80 fl has been in use. This would detect all α^0-thal individuals, and most α^+-thal subjects. Of 150 subjects with α^+-thal, 22 were found to have MCV $>$80 fl (range 80.2—84.4; mean 81.4 $\pm$ 1.13 fl) (unpublished data).

It is also possible to use mean cell haemoglobin (MCH) as an index for screening. This has an advantage over MCV as red cells stored at room temperature tend to swell with time. In a study on archived samples from 255 school children, Ma et al.[13] found that an MCH of $<$27 pg could effectively detect all α^0-thal and β-thal carriers. The presence of occasional H inclusions (around 1 in 1000 red cells) after incubation of red blood cells with 1% brilliant cresyl blue is an additive screening test for α^0-thal. Brilliant cresyl blue dye both precipitates and stains β-chain tetramers — β4 — which are present in a small proportion of α-thal cells. The precipitated Hb is easily visible under light microscopy by its 'golf-ball'-like appearance. In our experience, almost 100% of α^0-thal patients have occasional H inclusions with low MCV,[10] whereas β-thal carriers (patients with β-thal minor) have an increased HbA_2 level ($>$3.5%) in the presence of low MCV. However, the sensitivities of both these tests, namely occasional H inclusions and HbA_2 by microcolumn chromatography, would be markedly reduced in patients with iron deficiency, who would also have microcytosis. Thus, it would be necessary to exclude co-existing iron deficiency in all patients of the antenatal screening programme. If a person is found to be iron deficient, then MCV should be repeated after 4 weeks of ferrous sulphate therapy. Iron deficiency is rare in developed countries, and would account for no more than 1% of low MCV identified in the antenatal screening programme.[10]

As an alternative to the HbH inclusion test, it is possible to detect the embryonic ζ chains, that are present in erythrocytes of individuals with α^0-thal, by immunocytochemical staining.[14] A comparison between the HbH inclusion test and immunostaining with murine monoclonal antihuman ζ-globin chain showed that whereas all 58 cases of α^0-thal have positive H inclusion bodies, only 56 were found to express ζ chains in peripheral blood red cells, giving a specificity of 96.7% for the latter technique.[14] The relative cost of the latter is also somewhat higher than HbH inclusion test.

Individuals with compound α- and β-thal traits are present in populations in which both α- and β-thals are common. These individuals have low MCV ($<$80 fl) but HbH inclusions might be absent, whereas the Hb A_2 level is invariably raised. DNA analysis for α^0-thal is necessary for diagnosis. Thus, for couples in which one person has α-thal and the other β-thal traits ($\alpha\beta$-thal couples), it would be necessary to exclude α^0-thal in the partner who is a β-thal carrier.

Only couples who are both α^0-thal carry a 25% risk of having fetuses affected by homozygous α^0-thal (Hb Bart's hydrops fetalis) in each pregnancy. Most α^+-thal individuals can therefore be excluded from further investigation, unless the other partner has an α^0-thal genotype. Under this circumstance, it would be necessary to investigate the α^+-thal individual for non-deletion defects that are known to give rise to Hb H hydrops fetalis.[15—17] For deletional forms of α^+-thal and α^0-thal, such fetuses are fully compatible with life and adults with deletional HbH disease (three α gene deletions) only manifest a thal intermedia phenotype.

Our current practice of thalassaemia screening is carried out upon antenatal booking at public hospitals and maternal and child health centres. Education and information about thalassaemia is provided in the form of pamphlets, video display, posting on a website and explanation by nurse/counsellors. An example of a current screening programme is given in Fig. 1.

For a comprehensive prevention programme, screening for thal traits could be offered to teenagers at school or to those attending premarital counselling clinics. In 1990 in Hong Kong, screening was extended to couples attending the Family Planning Association (FPA) throughout the territory for premarital counselling. If both partners are found to have α^0-thal or β-thal minor, they are counselled about the mode of inheritance and management of thal. Thus they are forewarned about the necessity for prenatal diagnosis before marriage and well before pregnancy. In 1993, of 19,182 persons attending the FPA, 1427 (7.4%) were found to have a low MCV. However, only 39 couples had a low MCV in both partners and were referred for further DNA analysis. Of these couples, 18 were found to have α^0-thal, one had β-thal minor and 20 were couples with disparity of α- and β-thals. Only the first two categories require prenatal diagnosis.[18]

Invasive prenatal diagnosis

Prenatal diagnosis of fetuses at risk for homozygous α-thal relies on demonstrating the absence of α-globin chains or α-globin genes. The former was made possible with the introduction of fetal blood sampling in 1974.[19,20] Fetal blood is obtained either by fetoscopy or by direct aspiration from the placenta. The former yields relatively pure fetal

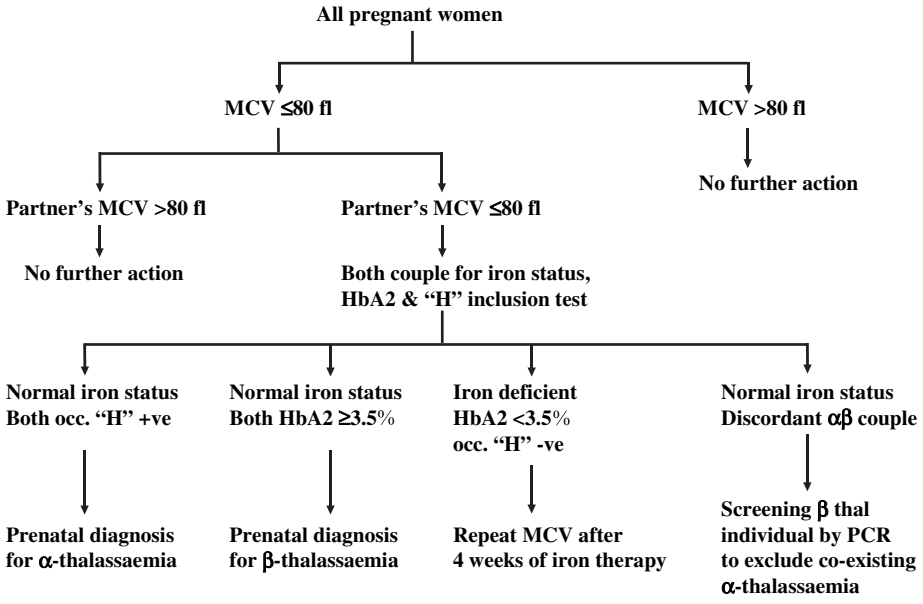

Figure 1 Flow chart of thalassaemia screening in Hong Kong (adapted from Ref. 10).

cells whereas the mixed sample from the placental aspiration requires Orskov lysis of the maternal cells.[21] After incubation of the fetal cells with radioactive amino acids, the absence of α-globin-chain synthesis in homozygous α^0-thal can be demonstrated.[22]

As fetal blood sampling carries a 5.6% fetal mortality rate,[23] and as α^0-thal — and particularly the southeast Asian type ($^{-SEA}$) — involves gene deletion, DNA analysis can be employed. This requires the extraction of fetal cells from amniotic fluid. Amniocentesis can be performed at 16—22 weeks' gestation with only 0.5% miscarriage risk to the fetus, which is close to the spontaneous miscarriage rate at mid-trimester.[24] Initially, the cDNA—DNA liquid hybridization technique was used to quantitate the number of α genes present.[25] This required a relatively large amount of fetal DNA, and hence culturing of amniocytes, which took approximately 14 days. In addition to the lengthy period required, the possibility of contamination of the cell culture and of α-cDNA with non-α-cDNA might lead to non-specific hybridization.[25]

The advent of restriction enzymes and of the Southern blotting[26] technique made it possible to visualize differently sized DNA fragments due to gene deletion. Direct analysis of fetal DNA is possible. Initially, the two sources of fetal DNA were uncultured amniocytes[27] obtained by amniocentesis at 18—20 weeks' gestation or chorionic villus from sampling (CVS) at 9—11 weeks' gestation. Although CVS has the advantage of a first-trimester diagnosis, the risk to the fetus is higher (1.0% miscarriage). It is expected that at established prenatal diagnosis clinics, where experienced obstetricians are performing the sampling procedures, fetal loss would be much reduced.

Southern blotting and gene mapping have been the gold standard for prenatal diagnosis, but recently specific amplification of the normal and the α^0-thal alleles by polymerase chain reaction (PCR) has become the method of choice.[28] Whereas PCR enables a more rapid diagnosis to be made (within a few hours of fetal sampling, thereby reducing waiting time and anxiety for the couples at risk), its sensitivity greatly compromises its specificity. Misdiagnosis can occur due to contamination of maternal decidua in the CV or as a result of allele dropout (ADO).[29,30] It is known that, with PCR, as little as 0.1% contamination with maternal or normal DNA will give an amplification of the 'normal' allele and lead to a false-negative result. The analysis of short tandem repeat (STR) in the same fetal sample can be used to exclude maternal contamination.

An alternative method is the quantitative PCR (Q-PCR) technique based on the TaqMan technology.[31] This enables the quantification of gene number because a fluorescent reporter dye is released with the synthesis of every new DNA strand. The TaqMan probe anneals internally on the DNA template between the 5' and 3' primers and serves to monitor the amount of PCR amplification. The technique is a real-time assay of PCR product accumulation and does not require post-PCR sampling handling, preventing contamination due to PCR product carry-over. As with the standard PCR protocol, specific amplifications of both the normal α allele and α^0-thal allele are made. Even with the deliberate contamination of an affected hydrops fetalis sample with 5% α^0-thal DNA (i.e. maternal DNA), there was no overlap in the result.[31] Thus, contamination should not pose any problem in this Q-PCR system, as >5% contamination of the CV by unseparated maternal decidua is an unlikely event in any experienced prenatal diagnosis laboratory. Although the Q-PCR system was established with primers for the detection of the $^{-SEA}$ type of α^0-thal deletion, using the Primer Express software it will be possible to design primers for amplification of other common α^0-thal alleles ($^{-Thai}$, $^{-Fil}$, $^{-Med}$ and $^{-(\alpha)20.5}$).[32]

As the 5' primer for the detection of the α^0-thal allele lies between the $\psi\zeta$ and $\psi\alpha_2$ genes, prior to the 5′ breakpoint region of the α^0-thal ($^{-SEA}$), the method has the advantage of being able to detect cases with ζ-α-thal deletion. The loss of the entire ζ-α-thal cluster is usually masked in a standard gene map, because no abnormal fragments will be generated by restriction enzyme digest. With the Q-PCR system, ζ-α-thal can be distinguished because of the variation in gene number between the normal α allele and the α^0-thal allele. Whereas the incidence of ζ-α-thal deletion is very low, when co-inherited with α2 codon 30 deletion, it will result in HbH hydrops fetalis,[16] possibly due to fetal anaemia or 'anoxia' in the early embryonic stage.

Non-invasive prenatal diagnosis

With an experienced hand and a good ultrasound machine, a non-invasive approach consisting of serial two-dimensional ultrasound examinations is effective at reducing the need for invasive testing in the majority of unaffected pregnancies.[33–35] We studied 832 at-risk pregnancies, 168 (20.2%) of which were affected. Fetal cardiomegaly and/or placentomegaly were present in these affected pregnancies.[35] The overall sensitivity and specificity of the non-invasive approach were 100% and 95.6%, respectively. The major benefit in the use of this non-invasive approach was the avoidance of an invasive test by about 75%[35]; the cost saving was relatively small in comparison to the cost of the whole prenatal screening programme for thalassaemia.[36]

The principle of the non-invasive approach is to detect ultrasonographic features of fetal anaemia. As α-globin-dependent haemoglobin F is the major haemoglobin of a fetus from 8 weeks' gestation onwards, anaemia can occur in an affected fetus after this time.

The non-invasive approach can be offered as an alternative for all women with an at-risk pregnancy. The approach is applicable in singleton as well as twin pregnancies,[37] and can be used to confirm normality in pregnancies conceived after pre-implantation genetic diagnosis (PGD).[38]

The non-invasive approach can be used as early as 12 weeks' gestation.[35] If an optimal view of the fetal cardiothoracic ratio (CTR) cannot be obtained by abdominal scan, visualization of the fetal heart can be improved by vaginal ultrasonography. However, at 10–11 weeks' gestation, an optimal fetal CTR could be obtained in only half of women even though vaginal scan was performed (unpublished data). There were no significant differences in the ultrasonographic measurements obtained at these gestations between the affected and unaffected pregnancies.

Several ultrasonographic parameters, including CTR, placental thickness (PT) and middle cerebral artery peak systolic velocity (MCA V_{peak}), have been investigated. In an affected pregnancy, the ultrasonographic measurements of the CTR, PT and MCA V_{peak} are increased because of severe fetal anaemia.[33,34,39–44]

The CTR is the ratio of the fetal transverse cardiac diameter taken at the level of the atrioventricular valves between the epicardial surfaces at diastole with the transverse fetal thoracic diameter.[33] An abdominal 5-mHz or 7-mHz curvilinear transducer or vaginal 5-mHz or 7-mHz vector transducer can be used. The optimal view is a subcostal four-chamber heart view rather than an apical view.

PT is a measurement of the maximal placental thickness, with the transducer placed perpendicularly to the placenta and measurements taken in the longitudinal and transverse sections.[40]

The predictive values of these parameters vary with gestation. At 12–15 weeks' gestation, the predictive values for the CTR were better than those for the PT.[35] Measurement of PT can be inaccurate if the placenta is adjacent to a focal myometrial contraction or located in the fundus or lateral uterine wall.[39] The placenta might be large but its thickness can be normal in an affected pregnancy.[39] MCA V_{peak} was not predictive at 12–13 weeks' gestation because of extensive overlap of its values between affected and unaffected fetuses.[44]

The predictive value of the CTR decreases with gestation age.[35] In advanced gestation, hydropic signs including ascites or pleural effusion are more apparent than cardiomegaly in affected pregnancies.[35] Measurement of the MCA V_{peak} might be a more sensitive sonographic parameter in identifying affected fetuses.[37,45,46]

It seems that three-dimensional assessment of placenta is no better than two-dimensional ultrasound in the first-trimester prediction of affected pregnancies.[47] Although the placental volume/crown–rump length ratio in affected pregnancies was larger than that in unaffected pregnancies, the difference was not significant.[47] The false-positive rate of this non-invasive approach was about 3% because disorders like intrauterine growth restriction, congenital heart disease[35] and HbH disease[48] can present with cardiomegaly and/or placentomegaly. An invasive DNA diagnosis is still recommended to confirm an affected fetus.

There is a risk of delaying the diagnosis of an affected pregnancy due either to lack of experience in the examination of the fetal heart on the part of the obstetrician or to suboptimal image resolution obtained at early second trimester.[35] The use of this approach demands an accurate measurement of the CTR. Adequate training and subsequent quality control are essential.[35]

In our practice, we offer all women at risk of carrying homozygous α^0-thal the option of the non-invasive approach. Serial ultrasound examination is performed at 12–15, 18 and 30 weeks' gestation. If there is fetal cardiomegaly (CTR $\geq$ 0.50, 0.52 and 0.59 at 12–15, 18 and 30 weeks' gestation, respectively) and/or placentomegaly (>18 mm at 12 weeks' gestation), CVS or amniocentesis for fetal DNA analysis, which is less invasive than cordocentesis, is offered.[35] With the use of Q-PCR, a rapid report can be available within 1–2 days of the procedure.[31] This time lag is comparable to cordocentesis for fetal haemoglobin pattern study. However, if ultrasound examinations are normal, an invasive procedure can be omitted. After birth, haemoglobin analysis of the cord blood is required to confirm an unaffected pregnancy. When the image quality of the fetal heart at 12 weeks' gestation is suboptimal, rescan in 2–3 weeks' time is a reasonable option if a woman still prefers ultrasound monitoring to an invasive testing.[35] The risk of delaying the diagnosis of an affected pregnancy until the second trimester, and the disadvantages of second-trimester termination, should be balanced against the risk of an invasive testing.

Other non-invasive prenatal diagnosis

A simple non-invasive technique for early detection of Hb Bart's hydrops fetalis was reported using the analysis of fetal erythrocytes in maternal blood.[49] The principle of the technique is based on the fact that red blood cells from the affected fetus express ζ-globin and not α-globin. By contrast, erythroid cells from the mother who is a carrier of the southeast Asian type ($^{-SEA}$) deletion express mainly α-globin and a small amount of ζ-globin.[50] Maternal blood smears were prepared and co-stained with fluorescent-labelled antibodies for α-globin (red) and ζ-globin (green). Analyses were then performed by examining the smear under a fluorescent microscope. Most of the maternal cells on the smear showed only red fluorescent signal. Some maternal cells, or fetal cells not affected by Hb Bart's hydrops fetalis, showed both red and green fluorescent signals. Affected fetal erythrocytes gave only a green fluorescent signal. With this dual immunofluorescent staining, erythrocytes from affected fetuses were detected as early as 8 weeks' gestation. As manual processing of slides is both tedious and time consuming, automated slide reading is being investigated such that more slides per case could be examined to yield better scoring results with efficiency.

It has recently become possible to use a multiplex quantitative fluorescent-PCR (QF-PCR) test on cell-free fetal DNA in maternal plasma to detect Hb Bart's hydrops fetalis.[51] Two polymorphic microsatellite markers within the southeast Asian type ($^{-SEA}$) deletion breakpoints are amplified using QF-PCR. Complete absence of these markers suggests a deletion on both alleles. The polymorphic nature of these microsatellite markers enables differentiation between maternal and paternal alleles, enabling the exclusion of maternal contamination by confirming the absence of non-inherited maternal alleles within the fetal DNA samples.

Pre-implantation genetic diagnosis (PGD)

Conventional prenatal diagnosis, whether DNA-based or by ultrasonography, can be performed only at 11—18 weeks' gestation. The decision to terminate an affected pregnancy is often difficult for the parents, particularly in second-trimester diagnosis; others, for religious or moral reasons, might not wish to terminate a pregnancy. In addition, couples with fertility problems, or a history of repeated elective terminations following prenatal diagnosis of affected pregnancies, might want to consider pre-implantation genetic diagnosis (PGD) as an option, as only embryos diagnosed as unaffected will be used to establish pregnancy.[52] Females undergoing PGD need hormonal treatment to induce the production of many ova, which are then harvested for *in vitro* fertilization (IVF). To ensure a single copy of paternal DNA in the PGD analysis, IVF is performed by intracytoplasmic sperm injection (ICSI). The embryos are cultured for 60 h to the eight-cell stage. Two single blastomeres are biopsied from each embryo and placed into separate Eppendorf tubes for genetic diagnosis.

Although there are over 200 reported cases of PGD for β-thal, the few reports for PGD of α-thal are mainly feasibility studies using either blastomeres obtained from mouse embryos or discarded human embryos from IVF clinics.[53,54] The success rate of PCR in amplifying the α-globin genes might be lower in cells from abnormal embryos[53] than in blastomeres obtained from fresh human embryos. A successful isolated case of PGD for α-thal was reported by Li et al.[55]

Chan et al. recently reported a larger series.[38] PGD of α-thal involves an initial multiplex gap-PCR of the normal allele and the α^0-thal allele, followed by individual semi-nested PCR for the two different alleles, using the first PCR product as template. The advantages of a second semi-nested or nested PCR are to ensure specific amplification and increase the amount of final PCR product. The sensitivity of detecting small amounts of final PCR products had also been improved with the use of a 5′ fluorescent-labelled primer and subsequent analysis of the fluorescent-PCR product by laser using the Genescan.[38,54]

In the current PGD protocol, the use of two blastomeres biopsied at the six- to eight-cell stage of the embryo prevents misdiagnosis due to ADO, as a true diagnosis was assumed only when concordant results were obtained from both blastomeres of the same embryo. ADO has always been a major concern in single cell PCR.[56—58] Various methods, such as isothermal whole-genome amplification[59] and multiple displacement amplification[60] on a single cell, have been introduced to enrich the amount of DNA for the simultaneous analysis of the molecular defect; polymorphic repeat sequence to monitor ADO or extraneous DNA contamination and aneuploidy testing. However, when only single cells were used, preferential amplification of one allele resulting in ADO was noted in 5—10.2% of cases. Hence, confirmatory diagnosis from two blastomeres is advocated, as it is less likely that the same allele would dropout in both analyses.

It is well known that removal of one or two blastomeres at the eight-cell stage from each embryo does not adversely affect human pre-implantation development *in vitro*.[61] It is almost 17 years since the first PGD. At some stage, data regarding the postnatal development of babies born after PGD should become available. Unfortunately, the removal of two blastomeres from a single embryo is highly dependent on the developmental state of the embryo on day 3 of culture. For PGD of homozygous α^0-thal, it is possible to further safeguard or circumvent misdiagnosis by only transferring embryos diagnosed as having at least one normal α allele.

Given that PGD is offered to α^0-thal couples who have undergone repeated terminations of affected pregnancies, as well as at-risk couples who are undergoing IVF treatment because of infertility, the pregnancy rate is low 15.4%. In a report that discusses PGD for β-thal, the pregnancy rate for infertile couples was 11.1%, compared to 30.8% for the fertile patient group.[62] The VIth data collection of the European Society of Human Reproduction and Embryology (ESHRE) PGD consortium reported 2984 cycles from 50 centres, up to 2003, and the overall clinical pregnancy rate was 16.7%.[63] Among PGD cycles initiated for single gene disorders, the most common autosomal recessive disease were cystic fibrosis, β-thal (with or without HLA typing), spinal muscular atrophy and sickle-cell anaemia; unfortunately, no α^0-thal cases were included. There

were common autosomal dominant diseases, such as Huntington's disease, myotonic dystrophy, adenomatous polyposis coli and Marfan's syndrome, as well as X-linked diseases like Duchenne and Becker muscular dystrophy, fragile X and haemophilia. Overall, 20% of cycles that reached the stage of oocyte retrieval resulted in a clinical pregnancy (a 27% clinical pregnancy per embryo transfer).

Verlinsky and Kuliev[64,65] advocated the analysis for aneuploidy in addition to PGD for single gene defect based on the assumption that, in many instances, failure of the 'unaffected' embryo to implant may be due to its inherent chromosomal abnormality. They reported an increase of 7% live-births following transfer of aneuploidy-free zygotes.[64] To achieve PGD for monogenic disorder and aneuploidy screening, one would need three cells from each embryo for analysis. Verlinsky and co-workers[64] used two polar bodies and a single blastomere for the purpose. However, using a single blastomere for examination of chromosomal abnormalities does not exclude the chance of mosaicism in the remaining cells of the embryo.[66] Indeed, misdiagnosis due to mosaicism has been confirmed in one case at delivery.[63]

In the ESHRE VIth database, follow-up of deliveries up to October 2004 revealed no increased incidence of pregnancy complication or malformation at birth in the PGD population.[63] The main complication was multiple pregnancies, sometimes leading to mortality or morbidity in the PGD offspring. This could be ascribed to the multiple pregnancies rather than to the PGD procedure. Data on the live-born children also showed good Apgar scores in 242 out of 255 babies and poor scores in 13 babies only. It would appear that PGD is an acceptable modality in offering at-risk couples unaffected children and avoids the need for termination of pregnancy.

In utero and postnatal treatment

A small number of children with homozygous α^0-thal have survived as a result of intrauterine blood transfusion or exchange transfusion at birth.[67,68] However, nearly half were found to have neurological and/or developmental problems. Furthermore, like all transfusion-dependent thalassaemia patients these children would require chelation therapy for their iron overload. Potential curative treatment includes in utero or postnatal haematopoietic stem-cell transplantation.[69]

Practice points

- MCV $<$ 80 fl is a useful screening tool for α^0-thal carriers.
- Couples who are both α^0-thal carry a 25% risk of having a fetus affected by homozygous α^0-thal in each pregnancy.
- Southern blotting and PCR analysis of fetal DNA obtained from CVS or amniocentesis are the mainstay for prenatal diagnosis of homozygous α^0-thal.
- Non-invasive prenatal ultrasound diagnosis from 12 weeks' gestation is possible by measuring the fetal cardiothoracic ratio, placental thickness and middle cerebral artery peak systolic velocity (later gestation).
- Pre-implantation genetic diagnosis is possible, particularly for couples with repeatedly affected pregnancies.

Research agenda

- To further develop non-invasive prenatal diagnosis using fetal erythrocytes and DNA in maternal blood.
- To study the option of in utero haematopoietic stem-cell transplantation.

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