Leading Article

Prenatal diagnosis

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Introduction

Prenatal diagnosis is a rapidly evolving branch of medicine. From its beginnings in the 1950's with the discovery of the human karyotype by Tjio and Levan, improvements in tissue sampling and cell cultivation techniques and the development of ultrasonic procedures have vastly increased the methods available for prenatal diagnosis. Today there is a wide range of non-invasive and invasive examination methods for pregnant women to choose from.

Non-invasive methods, based on obstetric ultra-sound examination and maternal serum biochemistry, are offered as screening tools for all pregnancies. Invasive methods (chorionic villus sampling, amniocentesis and fetal blood sampling), which aim at prenatal genetic diagnosis, are offered in selected high-risk pregnancies due to the quantifiable risk involved in all available methods.

Prenatal screening has become an accepted component in the practice of obstetrics over the past few decades. Most elements of standard prenatal care are relatively straightforward and easy for patients to understand and accept, but screening and diagnostic testing for chromosomal abnormalities remain confusing, emotionally charged and fraught with uncertain risks. Therefore, adequate counselling before performing prenatal diagnostic techniques is of paramount importance.

Ultrasonography and prenatal diagnosis

As a result of improvements in image quality and scanning technique, ultrasound now plays a central role in the prenatal detection of the abnormal fetus. The aim of this method is to reliably diagnose or, in the vast majority of cases, to exclude the presence of anomalies. Ninety-eight percent of routine ultrasound scans serve to reassure parents and therefore reduce much anxiety.

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Likelihood of detecting a fetal anomaly is related closely to gestational age at the time of procedure, equipment used and level of experience of person performing the scan. With recent technological improvements such as high-resolution ultrasound transducers fetal morphology can be assessed accurately at earlier gestational ages.

Although detection rates of up to 60% have been reported in late first and early second trimester routine screening by transvaginal or transabdominal route[1], second trimester ultrasonography remains the technique of choice for most indications. When first and second trimester examinations are combined, the detection of structural abnormalities is about 80%[2,3]. Some fetal defects evolve or progress in terms of their ultrasonographic characteristics as pregnancy progresses. Thus, certain defects cannot be excluded reliably by a single scan, particularly if performed in early pregnancy.

Definition of an optimal gestational age for prenatal diagnosis is dependent on the intention of screening. First and early second trimester scans are useful for high-risk pregnancies to reassure parents of absence of major malformations or to evaluate the choice of terminating a pregnancy with severe malformations. If the aim is to improve neonatal outcome, ultrasound screening at mid gestation or later in pregnancy is advisable.

Early pregnancy screening

First trimester screening methods include maternal serum biochemistry and the early US scan (11-14 weeks). First trimester screening offers several potential advantages over second trimester screening.

When test results are negative, it may help reduce maternal anxiety earlier. If results are positive, it allows women to take advantage of first trimester prenatal diagnosis by chorionic villus sampling (CVS) at 11-12 weeks or second trimester amniocentesis (15 weeks). Detecting problems earlier in the pregnancy may also allow women to

prepare for a child with health problems and affords greater privacy and less health risk if they elect to terminate the pregnancy.

Maternal serum biochemistry in first trimester

Maternal serum free ß-human chorionic gonadotrophin (hCG)

Maternal serum free ß-hCG normally decreases with gestation after 10 weeks. In trisomy 21 pregnancies, the levels are increased and the difference between these and the levels seen in normal pregnancies increases with advancing gestation.

Maternal serum PAPP-A

Maternal serum PAPP-A normally increases with gestation. In trisomy 21 pregnancies, levels are lower but difference between trisomy 21 and normal pregnancies decrease with advancing gestation.

Other maternal serum markers such as pregnancy-specific ß-1 glycoprotein (SP1), alpha-fetoprotein (AFP) and inhibin-A do not provide a useful distinction between affected and normal pregnancies in first trimester[4].

Early scan (11-14 week scan)

The early pregnancy scan was initially introduced with the primary intention of measuring the fetal crown-rump length to achieve accurate pregnancy dating. During the last decade however, improvements in the resolution of ultrasound machines has made it possible to describe normal anatomy of fetus and diagnose or suspect the presence of a wide range of fetal defects in the first trimester of pregnancy. As with introduction of any new technology into routine clinical practice, it is essential that those undertaking the 11-14 week scan are adequately trained and their results subjected to rigorous audit.

List of abnormalities detected at early transvaginal scanning (11-14 weeks) is growing rapidly. Reduced resolution of early US examination in relation to small fetal anatomy may not allow the detection of some cardiac, skeletal and gastrointestinal anomalies. Therefore, early screening by transvaginal sonography should always be followed by a mid-gestation transabdominal scan, as an additional 20% of malformations will be evident at that time[2].

Nuchal translucency thickness

In 1866, Langdon Down reported that the skin of individuals with trisomy 21 appears to be too large for their bodies. In the 1990's it was realized that the excess skin of individuals with Down syndrome could be visualized ultrasonically as increased nuchal translucency (NT) in the first 3 months of intrauterine life. Fetal NT at the 11-14 week scan has been combined with maternal age to provide an effective method of screening for trisomy 21. Recent evidence suggests that maternal age can be combined with fetal NT and maternal serum biochemistry (free ß-hCG and PAPP-A) at 11-14 weeks to identify about 90% of affected fetuses[5].

In addition to trisomy 21, increased NT can also identify a high proportion of other chromosomal abnormalities and is associated with major defects of the heart and great arteries, a wide range of skeletal dysplasias and genetic syndromes. Possible mechanisms for increased NT include heart failure, venous congestion of the head and neck due to superior mediastinal compression, altered composition of the extracellular matrix, abnormal or delayed development of the lymphatic system, failure of lymphatic drainage due to impaired fetal movements, fetal anaemia or congenital infection.

Since NT increases with crown-rump length, it is essential to take gestation into account when determining whether a given translucency thickness is increased or not. The optimal gestational age for measurement of NT is 11-13+6 weeks. Success rate for taking a measurement at this gestation is 98-100%, falling to 90% at 14 weeks[6]. NT can be measured successfully by transabdominal route in about 95% of cases. In the others it is necessary to perform transvaginal ultrasonography. In a study involving more than 100,000 pregnancies, the median NT increased from 1.2 mm at 11 weeks to 1.9 mm at 13+6 weeks[7]. Appropriate training, high motivation, adherence to a standard technique as well as use of good quality equipment and allocation of adequate time for each examination are prerequisites for a satisfactory NT measurement.

One-stop clinics for early assessment of fetal risk

An important development in biochemical analysis is the introduction of a new technique (random access analyzer using time-resolved-amplified-cryptate-emission), which provides automated, precise and reproducible measurements within 30 minutes of obtaining a blood sample[5]. This has made it possible to combine biochemical and ultrasonographic testing as well as to counsel in one-stop clinics for early assessment of risk (OSCAR).

Nuchal translucency followed by second trimester biochemistry

At 16 weeks of gestation, the median maternal serum concentrations of AFP, oestriol, hCG (total and freeß) and inhibin A in trisomy 21 pregnancies are different from normal. The risk of trisomy 21 is increased if the levels of hCG and inhibin A are high and the levels of AFP and/or oestriol are low. The estimated detection rates are 50-70% for a screen positive rate of about 5%. In women having second trimester biochemical testing following first trimester NT screening (with or without maternal serum biochemistry), the background risk needs to be adjusted to take into account the first trimester screening results.

Triple test

Current maternal serum testing in the second trimester uses three distinct hormones to screen for trisomy syndromes (trisomy 21 and 18) in low risk patients, while incorporating the detection of neural tube defects (NTD). The hormones tested are AFP, hCG and unconjugated oestriol. It is most accurate if performed between 16-18 weeks of gestation, but can be done from 15-22 weeks. Triple screening should not replace amniocentesis or chorionic villous sampling in pregnancies at high risk for trisomy 21. If amniocentesis is omitted in the care of pregnant women older than 35 years, 20-30% of fetuses affected by trisomy 21 will be missed with the triple test screening method.

Mid-gestation scan (18-23 weeks)

This scan systematically examines fetus for major and minor defects. Second trimester routine ultrasound scan should include

- Skull – examination of integrity and normal shape, measurement of biparietal diameter and head circumference.
- Brain – examination of cerebral ventricles, choroid plexus, mid brain, posterior fossa (cerebellum and cisterna magna), and measurement of anterior and posterior horns of the lateral ventricles.
- Face – examination of the profile, orbits and upper lip.
- Neck – measurement of nuchal fold thickness.
- Spine – examination both longitudinally and transversely.
- Heart – examination of rate and rhythm, four-chamber view and outflow tracts.
- Thorax – examination of shape of the thorax, the lungs and diaphragm.
- Abdomen – examination of the stomach, liver, kidneys, bladder, abdominal wall and umbilicus and measurement of abdominal circumference.
- Limbs – examination of the femur, tibia, fibula, humerus, radius and ulna, hands and feet (including shape and echogenicity of long bones and movement of joints) and measurement of femur length.

Studies on the efficacy of second trimester screening for detection of fetal malformations differ widely. Differences in the populations studied (low or high-risk), type of defects reported (major only or both major and minor), gestational age range at examination, availability of postnatal examination of the newborn and follow up, and post mortem examination of aborted fetuses, still births and post natal deaths, as well as the more obvious differences due to higher levels of expertise, better equipment, longer examination times all contribute to these varying results.

Efficacy of screening in different organ systems

When comparing detection rates of different fetal anomalies, it is apparent that some defects are almost always detected while others are detected rarely. Anomalies with the highest detection rates are those of the genitourinary tract (85%), spine and central nervous system (85%), and the gastrointestinal tract (74%). The lowest detection rates are for cardiac defects (32%) and most muscular and skeletal anomalies (30%)[3,8,9].

Doppler ultrasound and fetal anomalies

Colour Doppler plays a vital role in the diagnosis of fetal cardiac defects and in the assessment of homodynamic response to fetal hypoxia and anaemia. In addition, several non-cardiac fetal malformations including placental malformations, renal malformations, intracranial arterio-venous fistulae, pulmonary hypoplasia, diaphragmatic hernia, hepatic angiomas, and fetal tumours can be diagnosed using colour Doppler. Colour Doppler enables not only visualization of blood flow but also movement of body fluids. This can be helpful in the diagnosis of cleft palate, duodenal stenosis or atresia and vesico-ureteric reflux.

Fetal Magnetic Resonance Imaging (MRI)

Fetal MRI is currently being developed as a non-invasive imaging technique in prenatal diagnosis. The higher resolution and clarity of the images make MRI a very attractive tool for this purpose.

Prenatal genetic diagnosis

Prenatal genetic diagnosis involves invasive testing and includes amniocentesis, chorionic villus biopsy and fetal blood sampling. More than 70% of all invasive prenatal examinations are performed to exclude chromosomal abnormalities. The key indications for this type of invasive testing are advanced maternal age, pathological ultrasound findings and known familial chromosomal aberrations.

Amniocentesis

Amniocentesis is the most important and most widely accepted invasive prenatal diagnostic technique and is usually performed between 15-18 weeks gestation. With a miscarriage risk of 0.5 – 1% it is one of the safest prenatal diagnostic procedures. The introduction of fluorescence in situ hybridization (FISH) during interphase has increased the value of amniocentesis, enabling exclusion or detection of the most common aneuploides in the human genome with the help of DNA probes for chromosomes 13, 18, 21, X and Y on uncultured cell material, the results generally being available in 24 hours[10].

Chorionic villus sampling (CVS)

CVS is increasingly used to exclude known familial genetic defects. The procedure is performed after 11 weeks of gestation and carries a miscarriage risk of 2-3%. An association between early CVS and fetal defects, especially limb amputations have been observed in a series of studies[11]. Therefore, CVS should be performed only by appropriately trained operators and after 11 weeks of gestation.

Fetal blood sampling (FBS) / Percutaneous umbilical blood sampling (PUBS)

This is performed transabdominally under ultrasonic guidance after 18th week of gestation. The procedure carries a low miscarriage risk (<1%) and allows exclusion of a large number of disorders. Analysis of fetal blood not only enables cytogenic examinations but also prenatal detection of infections, haemoglobinopathies, and a variety of single gene defects.

Preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD) was introduced at the beginning of the 1990s as an alternative to prenatal diagnosis, to prevent termination of pregnancy in couples with a high risk for offspring affected by a sex linked genetic disease. At that time, embryos obtained in vitro were tested to ascertain their sex, and only female embryos were transferred. Since then, techniques for genetic analysis at the single cell level, involving assessment of the first and second polar bodies from oocytes or blastomeres from cleavage stage embryos, have evolved[12]. Fluorescence in-situ hybridization (FISH) has been introduced for the analysis of chromosomes and PCR for the analysis of genes in cases of monogenic diseases. In vitro culture of embryos has also improved through the use of sequential media[13].

Non-invasive risk-free strategies for prenatal genetic diagnosis

Up to now the only reliable way of obtaining fetal material for prenatal diagnosis was by invasive techniques such as CVS and amniocentesis. As these procedures are associated with considerable risk to both mother and fetus, there is a growing demand for development of safe and effective alternatives. Isolation of fetal cells from maternal blood[14] and the discovery of cell free fetal DNA in maternal plasma and serum[15] have opened a new frontier for the analysis of fetal genetic characteristics.

Ethical considerations in prenatal diagnosis

The more sophisticated prenatal diagnosis has become over the last few decades, the more complex the ethical questions raised. Most issues raised have no universally acceptable answers. It is generally accepted that prenatal diagnosis aims exclusively at preventing serious risks to child health. Pre-test and post-test counselling, obtaining informed consent, maintaining confidentiality and helping parents in the decision that they have made are all essential services provided by a prenatal diagnostic centre.

Counselling

Counselling is an integral component of prenatal diagnostic process. Counselling should be non-directive and include all relevant information. Parents need to understand what screening tests are being offered and how they may affect them. The clinician needs to explain to the couple the conditions detectable by the screen, diagnostic tests available if the screen is positive, risk to mother and child of the test being performed, accuracy of the test, the limitations of the test and be ready to answer any queries the couple might have. It is important that parents understand that a positive or a negative result in a screening test is not an absolute indication that something is or is not wrong with their baby. Parents should be given adequate time to think and arrive at decisions about how they will respond to the results before the testing occurs. Some parents change their minds when faced with an abnormal result or as a result of counselling before further testing.

The benefit of prenatal diagnosis

As with most other high-technology medical procedures in prenatal diagnosis too, the question of cost versus benefit arises. The most profound psychological benefit of prenatal diagnosis is the elimination of fears of an anomaly for the vast majority of pregnant women, providing reassurance and reducing anxiety. Prenatal identification of a fetus with an anomaly allows planning the delivery in a centre with a well-equipped neonatal unit and therefore avoids the need for postnatal transfer. Most of the benefits however, are not quantifiable in monetary terms and includes the benefit of sparing families of children with complex congenital diseases with many social, psychological and emotional consequences.

References

1. Yagel S, Achiron R, Moshe R. Transvaginal ultrasonography at early pregnancy cannot be used alone for targeted organ ultrasonographic examination in high-risk populations. *Am J Obstet Gynaecol* 1995;**172**: 971-5.

2. Kurjak A, Kupesic S, Matijevic R. First trimester malformation screening. *Eur J Obstet Gynaecol Reprod Biol* 1999; **85**: 93-6.

3. Grandjean H, Larroque D, Levi S and the Eurofetus study group. The performance of routine ultrasonographic screening of pregnancies in the Eurofetus study. *Am J Obstet Gynaecol* 1999; **181**: 446-54.

4. Noble P L, Wallace E M, Snijders R, Nicolaides K H. Maternal serum inhibin-A and free b hCG concentrations in trisomy 21 pregnancies at 10-14 weeks gestation. *Br J Obstet Gyanecol* 1997; **104**: 367-71.

5. Spencer K, Souter V, Tul N, Snijders R, Nicolaides K H. A screening programme for trisomy 21 at 10-14 weeks using fetal nuchal translucency, maternal serum free ß-human chorionic gonadotrophin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynaecol* 1999; **13**: 231-7.

6. Whitlow B J, Economides D L. The optimal gestational age to examine fetal anomaly and measure nuchal translucency in the first trimester. *Ultrasound Obstet Gynaecol* 1998; **11**: 258-61.

7. Snijders R, Noble P, Sabire N, Souka A, Nicolaides K H. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal translucency thickness at 10-14 weeks of gestation. *Lancet* 1998; **351**: 343-6.

8. Levi S, Schaaps J P, De Harvey P. End-result of routine ultrasound screening for congenital abnormalities: The Belgian multicentric study 1984-92. *Ultrasound Obstet Gynaecol* 1995; **5**: 366-71.

9. Carrera J M, Torrents M, Mortera C. Routine prenatal ultrasound screening for fetal abnormalities: 22 years' experience. *Ultrasound Obstet Gynaecol* 1995; **5**: 174-9.

10. Eiben B, Trawicki W, Hammans W. Rapid prenatal diagnosis of anueploides in unclutured amniocytes by fluorescence in situ hybridization -Evaluation of 3000 cases. *Fetal Diagn Ther* 1999; **14**: 193-7.

11. Firth H V, Boyd P A, Chamberlain P et al. Severe limb abnormalities after chorion villous sampling at 56-66 days of gestation. *Lancet* 1991; **337**: 762-3.

12. Verlinsy Y, Kuliev A. Preimplantation genetic diagnosis. *Reprod Med Rev* 1999; **7**: 1-10.

13. Sermon K, Van Steirteghem A, Liebaers I. Preimplantation genetic diagnosis. *Lancet* 2004; **363**: 1633-41.

14. Bianchi D W. Progress in genetic analysis of fetal cells circulating in maternal blood. *Curr Opin Obstet Gynaecol* 1997; **9:** 121-5.

15. Lo Y M D, Corbetta N, Chamberlain P F, Sargent J L. Presence of fetal DNA in maternal plasma and serum. *Lancet* 1997; **350**: 485-7.