First trimester prenatal diagnosis by chorionic villus sampling

The Johannesburg experience with 48 cases

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Summary

Chorionic villus sampling (CVS) is a first trimester alternative to amniocentesis for the prenatal detection of genetic disorders. Initial experience in 48 patients, in whom transcervical CVS was utilised for the diagnosis of chromosomal, biochemical or molecular disorders, is reported. An adequate villus sample was obtained in all cases and a diagnostic result was achieved in 90% of cases. In this series, the miscarriage rate was 4.2%. It is concluded that CVS appears to be a relatively safe and reliable procedure, but the risk of miscarriage can only be accurately assessed after further investigation.


Over the past decade and a half, amniocentesis has emerged as the mainstay of diagnostic procedures for the detection of genetic disorders and other birth defects. A number of studies have proved the safety and reliability of this technique. However, amniocentesis is not possible until the second trimester of pregnancy, necessitating a long anxious wait before investigations can begin and a further delay of up to 4-5 weeks until results are available. Furthermore, in the event of an affected fetus being detected, elective termination of pregnancy at approximately 20 weeks gestation is frequently accompanied by serious psychological stress and mid-trimester induced abortion is not free from medical risks and complications. It follows that the possibility of early diagnosis in the first trimester is much more acceptable to a couple 'at risk' for a genetic disorder; this explains the current widespread interest in developing first trimester diagnosis by chorionic villus sampling (CVS), and the increasing demand worldwide for this prenatal test.

First trimester CVS has a number of advantages: (i) the chorion is fetal in origin and is genetically identical to the fetus; (ii) the chorion is easily accessible during the first trimester of pregnancy either by a transcervical or a transabdominal approach; (iii) CVS offers several advantages to the parents (less delay in obtaining results; if necessary, simple aspiration termination of pregnancy; avoidance of the hazards of second trimester termination; and less bonding between fetus and mother at this early stage of gestation); and (iv) if inconclusive results are obtained on CVS, the patient still has the option of having an amniocentesis at 16 weeks' gestation.

Although CVS was first proposed by Mohr in 1968, it was not until relatively recently that the feasibility of this technique was illustrated. Since interest in CVS was reawakened some 4 years ago, there has been dramatic and rapid progress in the establishment of CVS as a routine technique for the monitoring of pregnancies at high risk of biochemical disorders, chromosomal abnormalities and disorders detected by DNA analysis.

Up to March 1988, more than 45 000 chorionic villus sampling experiences had been recorded worldwide. The associated mean miscarriage rate in the latter series of cases was 3.5%, compared with the 0.5-1% risk of miscarriage associated with amniocentesis between 15 and 17 weeks' gestation.

The first South African attempt at prenatal diagnosis by CVS was reported in 1985. Subsequent experience is now reported.
Patients and methods

Couples at risk for a genetic disorder and presenting before the 10th week of gestation were comprehensively counselled about the options in prenatal diagnosis. The pros and cons and technical aspects of CVS and amniocentesis were thoroughly discussed, as was our limited experience with CVS. Some experience in recognising suitable villus tissue had previously been acquired by the processing of a number of villus samples obtained, after informed consent, from patients undergoing legal first trimester terminations of pregnancy. Various overseas centres were also visited to obtain first-hand information of technical improvements. With full awareness of the latter factors, the majority of at-risk couples opted for CVS.

CVS procedure

The sampling procedure was undertaken at between 9 and 11 weeks' gestation, on an out-patient basis. Ideally, ultrasonography was performed 1 week before the sampling, at which time an endocervical swab was sent for microscopic examination and culture. Sensitivity screening included ureaplasma and for chlamydia infection. The procedure was performed with the patient in the lithotomy position without sedation. Initially, the cervix was grasped with a vulsellum in order to 'stabilise' it and decrease uterine mobility, but latterly this has usually been found to be unnecessary. A long cannula, preferably metal, inserted under ultrasonographic control into the extra-ovular cavity of the uterus at the thickest part of the developing trophoblast appeared to offer the best chance of success in terms of obtaining sufficient chorionic villi for laboratory analysis. Once the cannula was in position in the implantation site, suction was applied using a 20 ml syringe. Aspiration continued with a limited 'hoovering' action over a few millimetres along the path of insertion. This implies two or three backward and forward movements. Suction was discontinued as the cannula was withdrawn from the implantation site. The cannula was then removed carefully under ultrasonographic guidance.

Samples were aspirated directly into serum-free culture medium (Hams F10) or Hanks balanced salt solution with added heparin. The samples were immediately examined in the room where the procedure was performed macroscopically and/or microscopically for the presence of villi.

Laboratory procedure

The sample was processed in the laboratory within half an hour. In all instances, the sample was initially washed and then meticulously dissected under low microscopic magnification in order to separate the villi from contaminating maternal decidua. The 'clean' villous material was then processed according to the amount of tissue obtained and the genetic condition under investigation. However, when sufficient material was available, a cytogenetic analysis was always attempted in addition to the molecular or biochemical assays being carried out.

Molecular methods. In all instances requiring molecular testing by means of linked restriction fragment length polymorphisms (RFLPs), family studies were first performed and only if the family proved informative or partly informative, was CVS offered. A sufficiently large chorionic villus sample was obtained in all cases, circumventing the need to culture the tissue before extracting DNA. DNA was consequently directly extracted from fresh villi and thereafter analysed according to conventional methods.6

Biochemical methods. Three couples at risk for Tay-Sachs disease elected to have CVS. Chorionic villus tissue was washed in normal saline and thereafter subjected to two cycles of freezing and thawing and then sonicated in a small volume of saline. The sonicate was centrifuged and the supernatant used as an enzyme source. The hexosaminidases present were electrophoresed on Cellogel according to the method of Carmody et al.9 Hexosaminidase A and B activities on 4-methylumbelliferyl-N-acetyl-β-D-glucosamine were estimated by the heat inactivation method of O'Brien et al.,10 as modified by Kaback.11 Both heated and unheated enzyme solutions contained bovine serum albumin at a concentration of 0.6 mg/ml. In addition, hexosaminidase A activity towards 4-methylumbelliferyl-β-D-N-acetylglucosamine-6-sulphate (HSC Research Development Corp., Toronto, Canada) was determined according to the manufacturer's instructions.

Cytogenetic methods. When possible, the villus sample was divided into three portions to be used for: (i) direct analysis; (ii) 24-hour synchronised culture; and (iii) long-term culture. The direct method followed was very similar to that described by Simoni et al.,12 while fluorodeoxyuridine (FdU) synchronisation was carried out according to Gibas et al.,13 technique. In some instances, bromodeoxyuridine at a concentration of 30 μg/ml was supplemented instead of FdU.14 Long-term cultures were most successfully established after sequential enzymatic treatment with 0.0625% trypsin-EDTA and 100 U/ml collagenase (Jackson and Coutinho — personal communication, 1986). The long-term cultures were maintained in Chang medium without added fetal calf serum, which was found to give superior results compared with conventional culture media such as Hams F10 or RPMI, supplemented with fetal calf serum.

Results

In all 48 attempts at CVS, an adequate (>10 mg) villus sample was obtained, usually following two to three attempts at biopsy. To date, 2 of the patients who underwent CVS miscarried, giving a miscarriage rate of 4.2%. The indications for these prenatal diagnoses are listed in Table I. In summary, results relevant to the indication for CVS were obtained in 90% of cases. All 5 unsuccessful cases occurred early in the present series — cases 2, 4 and 5 failed to yield cytogenetic results, while a molecular diagnosis was unsuccessful in cases 10 and 22. A major problem was initially encountered with fetal karyotyping using cultured villi in that cytotrophoblast cells show a low growth potential under ordinary culture conditions. In this respect, the introduction of hormone-supplemented Chang medium had a markedly positive effect on cell growth.

<table>
<thead>
<tr>
<th>Indication</th>
<th>No. of cases</th>
<th>No. successful</th>
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<tbody>
<tr>
<td>Advanced maternal age</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Previous Down syndrome baby</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Chromosome-translocation carrier</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Tay-Sachs disease</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Haemophilia</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>β-thalassaemia</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sickle-cell anaemia</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>48</strong></td>
<td><strong>43 (90%)</strong></td>
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Discussion

Preliminary experience indicates that an adequate villus sample and results can be obtained in the great majority of cases. We have successfully used CVS for karyotyping and for the prenatal diagnosis of inherited disorders detectable in the fetus by biochemical analysis or by gene probing. However, neural tube defects and other congenital malformations detectable by maternal serum and/or amniotic α-fetoprotein estimation and ultrasonography can still be screened for only in the second trimester of pregnancy.

To date, 1 apparent misdiagnosis has been made in a pregnancy at risk for cystic fibrosis (CF). DNA was directly extracted from the CVS and was forwarded to a paediatric genetics centre in Britain that has considerable experience in the molecular diagnosis of CF. Before this, DNA samples from the parents and from a fetus aborted in 1986 on the basis of a positive microvillar enzyme test were sent to the same laboratory. A different pattern was found when the CVS-DNA was compared with the aborted fetal DNA. On this basis it was predicted that the present baby was unaffected, with the proviso that the diagnosis of CF was correct in the aborted fetus. After the premature birth of the female baby, it was found that the infant had meconium ileus, indicating probable CF.

The possibilities which could have resulted in this misdiagnosis include: (i) a cross-over between the molecular marker used and the CF gene; (ii) a sample mix-up; (iii) DNA was extracted from maternal tissue and not fetal tissue; (iv) a false-positive microvillar enzyme test on the first fetus; and (v) the present baby is affected by non-CF meconium ileus. These postulates are all at present being investigated in order to exclude a recurrence of such a probable misdiagnosis.

The obvious merits of CVS rest in the fact that it is performed in the first trimester of pregnancy and the time needed for obtaining results is usually much shorter than that following amniocentesis. A decision regarding the management of the pregnancy can be made within the first trimester, so reducing the emotional and physical stresses of reproduction for couples at genetic risk.

A major concern is that occasionally there have been discrepancies reported between the villus and fetal karyotypes, either in the form of mosaicism or maternal cell contamination, especially in cultured villi. In order to maximise the success rate, it is therefore essential that a meticulous technique for removing maternal cells be incorporated into the CVS protocol, and furthermore, both direct preparations and cultured preparations should, where possible, be analysed. In instances where the CVS analysis has yielded inconclusive results, the option to continue the pregnancy, followed by mid-trimester amniocentesis, should be considered.

The ultimate value of CVS will be determined by the risk to the pregnancy. However, this could prove difficult to measure because of the high background level of spontaneous abortion in the first trimester of pregnancy. Nevertheless, fairly reliable figures should soon be available, pending the results of risk evaluation studies based on pooled data from various centres abroad.1,2

In conclusion, our initial experience has shown CVS to be an acceptably safe and reliable method for prenatal diagnosis. Should it be demonstrated that the risk of fetal loss is low, approaching that of amniocentesis, CVS has the potential of becoming a viable and possibly preferable alternative to amniocentesis for prenatal diagnosis in the RSA.

We thank Dr Maxi Pinto for initiating this study in 1985. We are grateful to Professor Justus Hofmeyr for samples 4, 9 and 21 and in addition thank numerous private practitioners for referring their patients for CVS. We are indebted to Dr Maurice Super of Manchester for help with the molecular diagnosis in cases 7 and 13, and likewise to Dr Reuben Milbash of King’s College, London, for cases 11 and 19. Mr Jeremy Herbert of the cytogenetics laboratory of the Department of Human Genetics, University of Stellenbosch, performed the molecular diagnosis in case 12 and Mrs Colleen Morgan of our department carried out the required Tay-Sachs assays. We also gratefully acknowledge all the work carried out by the Molecular Genetics Unit of the South African Institute for Medical Research, Johannesburg.

Addendum

We have made a further 12 prenatal diagnoses using CVS. Successful results were obtained in all cases.

REFERENCES