Chorionic villus sampling for first-trimester fetal diagnosis: preliminary experience

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Chorion biopsy was performed in 110 cases at 6-12 weeks of pregnancy, following an ultrasonic chorion frondosum localization. 100 cases had their biopsies taken immediately before induced abortion and in 10 cases, biopsy was performed for genetic reasons. Chromosomal analysis by direct preparation was carried out in 108 cases. Enzyme assay was carried out in 40 cases for β-Galactosidase, α-glucosidase, α-galactosidase, β-glucuronidase and arylsulphatase. The possibility is discussed that the present approach may be developed for clinical use as an alternative to transabdominal amniocentesis in the 16th week.

Key words: Chorion biopsy; Prenatal diagnosis.

Recognition of genetic disease by prenatal diagnosis has become an increasingly important component of antenatal care. At present prenatal diagnosis is possible in the second trimester. These samples are subjected to cytogenetic and biochemical analysis. However the use of chorionic villi for early antenatal diagnosis of genetic disorders is rapidly gaining practical importance. Its main advantage over amniocentesis is that fetal tissue can be obtained without penetration of the fetal membranes. In addition it allows time for repeat sampling. We report here our experience with chorionic villus sampling emphasizing that direct chromosome analyses and enzyme determination can be carried out in the first trimester accurately for genetic disorders.

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Material and Methods

To perfect the technique of chorionic villus sampling, we asked 100 women scheduled for selective termination of pregnancy, at gestation ages 6-12 weeks to agree to the procedure for research purposes. Ten cases were taken up for diagnostic purposes for genetic indications. In cases requiring termination, biopsy was performed immediately before the termination. No anesthesia was administered during the sampling procedure. Gestational age was recorded from last menstrual period. The vulva, vagina and cervix were carefully swabbed with betadine solution; anterior lip of the cervix was grasped with a tenaculum; slight traction was exerted to reduce the angle of the cervical-uterine junction. The tip of a 1.5 mm oD 21 cm long catheter with malleable stylet (Portex) was inserted, the stylet withdrawn and villus material removed by repeated aspiration. In the diagnostic cases, this procedure
was carried out under real-time ultrasonic guidance. Following this, suitable antibiotics and uterine relaxants were prescribed for seven days. A follow-up scan was performed in all those cases who were to continue pregnancy.

**Chromosome preparations**

The aspirated samples were carefully inspected under an inverted microscope. Typical fragments showing good morphology were picked out with forceps and washed in Hank's balanced solution in petri dishes. The villi were then placed in 3 ml Ham's F10 medium containing colcemid (0.04 µg/ml) for 1 hour. It was then treated with 1% sodium citrate for 10-20 minutes and treated with fixative. The tissue was dissociated in 60% acetic acid. Slides were prepared as per usual protocol and 15 metaphases were scored and giemsa banded.¹

**Lysosomal enzyme determination**

The sample was immediately collected in physiological solution at 4°C, and dissected and cleaned. Samples after dissection were kept at -20°C. They were extracted by homogenizing the tissue with 0.25 ml saline and 0.25 ml Triton ×100. The homogenates were centrifuged at 15000 rpm for 30 minutes and the supernatant was submitted to assay. Protein was assayed according to the Lowry method.² Enzymatic activity was determined by fluorimetric techniques as described by Galijaard.³

**Results**

A total of 100 women who were undergoing therapeutic abortion for non-genetic reasons, consented to participate in the study. In most of the cases we were able to obtain 7-10 mg of villi (as estimated by visual comparison of the sample in photographs of weighed quantities) giving an overall success rate of 80%. By this method, cytogenetic technique took 2-3 days. However the banding quality following direct preparations was not as good as those obtained on culture. Thus we presume diagnosis of structural aberrations may be difficult.

In the 10 women who consented to diagnostic villi sampling, the indications for sampling were a previous child with trisomy and advanced maternal age. Villi were obtained in 8/10 cases however cytogenetic analysis could only be performed in 6/10. None of the patients encountered any bleeding. One of the mothers who had a previous Down Syndrome delievered a normal infant, as predicted.

The results of chromosomal analysis was as follows. In 2/10 aspiration did not contain villus. In these two biopsies tissue disintegration made morphology difficult. However it was noted that when the original tissue was highly budding, a good number of metaphases were obtained.

The mean activity of enzymes, number of samples tested and their ranges are given in the Table. All samples provided enough material for protein determination, however only 1-2 enzymes could be tested from each sample. The levels of enzymes appeared to be slightly higher than that obtained for leucocytes.

**Discussion**

Chorion frondosum is spread over the entire surface of the fetal sac at first, but soon its major part becomes smooth and chorion frondosum is only left where