Rapid prenatal karyotyping using foetal blood obtained by cordocentesis

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ABSTRACT

Background. Prenatal karyotyping using foetal blood samples obtained by cordocentesis is a useful method of detecting abnormal chromosomes in the foetus.

Methods. Cordocentesis was performed in 187 cases for prenatal karyotyping between January 1995 and September 2000. Pregnant women were between 18 and 38 weeks of gestation and their ages ranged from 18 to 40 years. The common indications were ultrasonographic abnormalities (47.6%), history of previous Down syndrome (13.3%), advanced maternal age (11.7%), low maternal serum alpha foetoprotein levels (10.7%), previous child with malformation (10.7%), previous child with trisomy (chromosome 13/18) (2.6%), parent balanced translocation carrier (1.6%) and high maternal serum alpha foetoprotein levels (1.6%).

Results. Analysis of 137 successful cultures showed 8 (5.2%) karyotype abnormalities. The remaining samples could not be reported due to the presence of maternal contamination of the sample (12.3%), inadequate sample (6.4%) or culture failure (9.8%). In those with an abnormal karyotype, obstetric management could be altered appropriately.

Conclusion. In foetuses at high risk of a chromosomal aberration, a rapidly obtained karyotype is helpful in obstetric management.


INTRODUCTION

Prenatal karyotyping using lymphocytes from cord blood is a useful method of detecting chromosomal errors in the foetus. It is usually advised in pregnancies in which an ultrasound or any laboratory finding suggests a foetal anomaly during the second trimester of pregnancy. Rapid advances in ultrasound have allowed evaluation and easy recognition of a foetal abnormality which needs karyotyping to rule out a chromosomal anomaly.1 A first or second trimester placental biopsy has also been suggested as a useful method but is labour-intensive and the metaphase spreads are difficult to interpret.2 Amniocentesis is the safest technique and can be carried out at 16–19 weeks of gestation. However, the culture takes about 2–3 weeks and may not provide the required information in time.3,4 Cordocentesis is usually advised between 18 and 22 weeks of gestation, although it can also be done in the third trimester of pregnancy. It is a reliable method for obtaining a foetal sample and lymphocytes can be cultured to prepare the karyotype in a short period of time.5

A substantial proportion of foetal abnormalities are associated with abnormal chromosomes. Swift ascertainment of foetal karyotype is required to facilitate future counselling, ensure proper obstetric management and in case of an affected foetus provide the option of termination of pregnancy. However, cordocentesis is technically difficult and carries a 2% risk of abortion.6

We karyotyped cord blood samples from January 1995 to September 2000 in 187 patients and present our experience.

METHODS

A total of 187 patients underwent foetal karyotyping using cord blood samples during this period. The gestational age of the women at the time of sampling ranged from 18 to 38 weeks and the maternal age ranged from 18 to 40 years. The common indications for cordocentesis were foetal malformations detected by ultrasound, a previous child with trisomy, advanced maternal age, low or high maternal serum alpha foetoprotein levels (MSAFP), a previous child with malformations or balanced translocation carrier parent. The ultrasound abnormalities included oligohydramnios, intrauterine growth retardation (IUGR), non-immune hydrops, neural tube defect, hydrocephalus, duodenal atresia or any other structural malformations of the foetus.

All patients were counselled prior to the procedure and the risks and benefits of cordocentesis explained. After an informed consent, 1–3 ml of foetal blood was obtained aseptically from the umbilical cord under ultrasound guidance using a 22/23 gauge needle. The commonest site of foetal blood sampling was the umbilical vein at the placental insertion of the cord about 1–1.5 cm away from the placenta. Other sites less commonly used were a free floating loop of the cord or foetal insertion of the cord. The needle tip was manoeuvred using the ‘needle guide’ or the ‘free hand’ technique.4 To rule out contamination with maternal blood, Kleihauer’s test was performed using adult blood and newborn blood samples as negative and positive controls.9

An aliquot of foetal blood was cultured in triplicate in 10 ml RPMI 1640 media supplemented with 15% foetal calf serum, antibiotic mix and stimulated with mitogen phytohaemagglutinin. The cultures were incubated at 37°C for 48–72 hours by the microculture method and then harvested.9 Chromosomes were prepared and fixed slides were treated by Giemsa–trypsin for banding metaphase chromosomes.10 In each case, at least 11 well spread metaphases were scored for detecting chromosomal abnormalities. In case of mosaicism or mixed samples, at least 50 metaphases were scored. The reports were provided to the families within a week of collection of the samples.

RESULTS

The most common indication for cordocentesis was an ultrasound abnormality in 89 cases (47.6%) followed by a history of previous Down syndrome in 25 (13.3%) and advanced maternal age in 22 (11.7%). Severe oligohydramnios (25.8%) was the most common indication on ultrasound followed by non-immune hydrops, renal malformation and IUGR (Table I). Thirty-nine per cent of the women were in the age group of 25–29 years and the gestational age in 35.8% women was 20–25 weeks. Pure foetal samples were obtained in 134 cases (71.6%), pure maternal samples in 23 (12.3%), while mixed foetal and maternal samples were obtained...
in 18 (9.6%). Twelve samples (6.4%) were not processed as they were clotted, haemolysed, inadequate or not fit for culturing. Therefore, 35 samples were excluded as they were inadequate (12) or maternal (23).

Cell cultures were set up for pure foetal and mixed samples (n=152). For mixed samples the karyotype was prepared and reported, mentioning the percentage of mixing of foetal and maternal blood. A normal 46,XX karyotype was found in 65 foetuses (42.7%) and 46,XY in 64 (42.1%; Table II). Due to culture failure or contamination, 15 samples (9.8%) could not be reported upon. An abnormal karyotype was found in 8 foetuses (5.2%) (Table III).

There was no foetal loss related to the procedure. Follow up cord blood karyotype could be done at birth in pregnancies where a normal karyotype was reported in about half the cases and was confirmed to be normal.

DISCUSSION

Our experience with cytogenetic evaluation of cord blood samples in a developing country shows that cordocentesis is safe, reliable and feasible for foetal evaluation of chromosomal disorders. It is beneficial in two clinical situations: (i) detection of a foetal anomaly in a patient at risk of chromosomal abnormalities before the time up to which the termination of pregnancy is legal; and (ii) when foetal anomalies are detected late in the third trimester when termination is not possible, but the knowledge of an abnormal karyotype would influence obstetric management. The main benefit is the ability of the technique to provide rapid and accurate results in patients with a late referral (after 18 weeks of gestation). The widespread use of ultrasound has resulted in increased antenatal detection of foetal abnormalities which require prenatal karyotyping. Cordocentesis is technically more difficult, has a higher risk and is carried out after 18 weeks of gestation. However, compared to chorionic villus sampling and amniocentesis it is more cost-effective and the reporting time is less. The post-procedure foetal mortality rate following amniocentesis is 0.5% while following cordocentesis it is 2.1%. In our group of patients there was no foetal death. In India, karyotyping is being done at many centres but facilities to culture chorionic villus and amniotic fluid for preparing the karyotype are available at only a few centres. Many mothers report late in pregnancy to obstetricians and may not have regular antenatal check-ups. Hence, foetal karyotyping is likely to be used more often in India.

Contamination of the sample with maternal blood is of concern because it limits the success rate. If the sampling is done too near the placenta the risk of admixture of maternal and foetal blood increases. Some of the failures in our cases were due to maternal obesity or oligohydramnios.

A number of methods to determine the purity of foetal blood samples have also been described based on haematological and biochemical indices such as blood typing, mean red blood cell
volume, erythrocyte i/I antigens, B-hCG and coagulation factor assay. However, we did not find any comparative studies. We found the Kleihauer’s-Betke test to be useful for confirmation of the source of blood collected during cordocentesis. It helped in scoring the foetal and maternal cells in the cord blood samples using adult and newborn samples as negative and positive controls.

Of the 187 samples received, successful chromosomal analysis was done in 137 cases (73.2%). In 35 cases (17.7%) blood was either of maternal origin or not adequate/fit for culturing. This is clinically important because examination of maternal rather than foetal cells could provide misleading cytogenetic results. Similarly, samples which were clotted, haemolysed or inadequate could not be cultured. For all mixed samples, the karyotype was prepared for both the cell lines and larger number of metaphases were scored (>50). If an XX/XY karyotype was found it was concluded that the foetus was a male, but in cases where all scored metaphases had an XX karyotype it indicated the presence of a female foetus. The percentage of mixing of maternal and foetal blood samples as detected by the Kleihauer’s-Betke test played an important role in determining the type of sample (maternal/foetal). In cases where there was mixing of foetal and maternal blood, the family was also counselled about contamination of the sample and the problems of providing a conclusive result. The culture failure rate due to bacterial contamination was 9.8%. Thus, the overall success rate of the procedure was less than that of reports from other countries where 90% success is seen. However, comparing the present results with those of a previous study from our centre carried out from 1990 to 1994, we observed an improvement in the overall success rate from 67.6% to 73.3%. There has also been an increase in the number of cases being referred for cordocentesis. The failure rates due to bacterial contamination in the previous study was 18% and has now decreased to 9.8%. This has decreased further to 5% in the last two years and could be the result of an improvement in laboratory conditions. In the previous study, maternal or inadequate samples comprised 14.2% of the total samples but are now 17.7%. This may be because the number of obstetricians doing the procedure at present are more than before. We also observed that the rate of maternal contamination had decreased from 20% in 1995–96 to 8% in 1999–2000.

We found 8 abnormal karyotypes (5.2%) which is similar to the results of the previous study from our unit. The low incidence may because most of the referrals were due to ultrasound abnormalities which are not uniformly related to a specific karyotype. Palmer et al. reported 27% abnormal unbalanced karyotypes in 107 pregnancies with ultrasound abnormalities. There are other reports with similar observations. The incidence of abnormal karyotypes in foetuses with isolated anomalies is about 5% but when multiple foetal anomalies are detected in utero by ultrasound, the risk of an associated abnormal karyotype rises sharply. All foetal structural malformations and cases of IUGR with or without severe oligohydramnios should be recognized as indications for karyotyping the foetus. This raises a dilemma as the majority of these cases are recognized at an advanced gestational age and the option for termination of pregnancy may not be available to the patient. Thus, all patients should receive appropriate counselling regarding the available options before undergoing cordocentesis.

REFERENCES