AMNIOCENTESIS FOR ANTENATAL DIAGNOSIS OF CHROMOSOMAL ABNORMALITIES AND NEURAL TUBE DEFECTS

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INTRODUCTION

AT SOME TIME during a pregnancy most women fear having a deformed or a mentally defective child. In most cases, sympathetic counselling by the obstetrician is sufficient to keep anxiety from developing to an abnormal extent. However, where a previous pregnancy has resulted in a chromosomally abnormal offspring or when there is a known genetic disease, a method has been available to demonstrate whether the fetus is normal or affected and a normal pregnancy allowed to continue or termination of pregnancy offered for an affected pregnancy. Midtrimester amniocentesis for antenatal diagnosis of a variety of genetic, developmental or metabolic diseases is now a practical procedure (Milunsky, 1973; Emery, 1973; and Goldman *et al.*, 1977).

Seventy-five cases undergoing amniocentesis for antenatal diagnosis of chromosomal disorders and neural tube defects are retrospectively and prospectively analysed.

MATERIALS AND METHOD

Clinical Methods

The patients were referred to the various consultant units at the Bradford Royal Infirmary and St. Lukes Hospital Maternity Services. Amniocentesis was offered whenever one of the indicators of increased risk was present. The procedure of amniocentesis and its related risks was explained to the patient. Trans-abdominal amniocentesis was performed under local anaesthesia whenever possible in the 14th to 16th week of pregnancy. An ultrasonic scan was performed prior to the procedure and towards the end of the study amniocentesis was performed under ultrasonic visualization. Under local anaesthesia and an aseptic technique a 19 gauge spinal needle was inserted at right angle to the uterine wall avoiding the placental site. The stylette was then withdrawn and the initial 1 to 2 ml. of amniotic fluid discarded, and then 20 to 30 ml. of fluid aspirated.

Selective abortion was performed when indicated by intra-or-extra-amniotic instillation of Prostaglandin F 2 alpha (PG F2α). The children who were ultimately delivered, however, were all examined by the paediatrician within 24 hours following delivery and again at the post-natal clinic.

Between 1972 and 1977, 76 patients had amniocentesis performed. Records of 72 patients were available for analysis. Three patients had amniocentesis performed in successive pregnancies, making a total of 75 pregnancies suitable for analysis.

Cell Culture Methods for Amniotic Fluid Cells

This involved centrifugation of the sample at full speed for 10 minutes and culture of cells in TC 199 medium enriched with AB+serum with added antibiotics (Penicillin and Streptomycin) in an environment of air enriched with 5% carbon dioxide at 37°C. After 10 to 14 days, cells were arrested in metaphase with colchicine. The chromosomes were stained with Giemsa and the karyotype decided after studying an average of 16 cells.

Determination of Alphafetoprotein in Amniotic Fluid

The supernatant was used to determine alphafetoprotein by the method of single radial immunodiffusion in 2% agarose gel containing 1.75% monospecific rabbit antiserum to human alphafetoprotein as previously described, (Stewart *et al.*, 1975).

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RESULTS

Ages of Patients and Indications for Amniocentesis

Table I shows the age distribution of the patients at the time of amniocentesis. Of the 75 patients, 26 were aged less than 35 years, and the majority, 49 patients, were aged 35 to 49 years. The highest proportion were in the 35-39 year age group and numbered 30 (40%)

Table I

Age distribution of patients undergoing amniocentesis

Age (years)	Number of Patients	%
20 — 24	8	(10.7)
25 — 29	14	(18.7)
30 — 34	4	(5.3)
35 — 39	30	(40.0)
40 — 44	16	(21.3)
45 — 49	3	(4.0)
Total	75	(100.0)

The amniocentesis was performed for the sole indication of maternal age of 30 years or more in 46 pregnancies (61.3%) and in 10 (13.3%) because of a history of a previous child affected by Down's Syndrome. Sixteen cases (21.3%) had it done because of a previous child affected by neural tube defect. In two (2.6%) there was a previous mentally retarded child where the precise diagnosis was not known and in another, because of a family history of Down's Syndrome and mental retardation. One patient requested the procedure because of anxiety of an abnormal fetus. In nine patients there was more than one indication for investigation.

Karyotype Results

The fetal karyotype was normal in 64 cases. In three cases it indicated a fetus affected by Trisomy 21 (Down's Syndrome) and these patients elected to have their pregnancy terminated. These patients were aged 39, 42 and 43 years respectively, in each the indication for the procedure was maternal age and the karyotype in all was 47 XXG+. The method of termination was by extra-amniotic Prostaglandin (PG F2α) in one case and by the intra-amniotic route in the other two cases.

In three cases anomalies of karyotype were reported. In none was the pregnancy terminated or a retap done. The first case had a karyotype reported as 46XX in 6 cells and 92XX in 2 cells, she delivered a normal male infant. The second case karyotype result was 46XX in 4 cells, 45XXB- in 2 cells and 45XXC- in 2 cells, a phenotypically normal female child resulted from the pregnancy. The third case had a karyotype of 46XX in 8 cells and 46XY in 12 cells and she was delivered of a normal male infant.

The sex of the child was unknown in 17 cases, 13 of which were undelivered at the time of writing and two cases where termination of pregnancy was performed. This included one case which was performed privately without the obstetrician's knowledge, and one in which the abortion was thought to be related to the amniocentesis.

Repeated Attempts at Amniocentesis

Repeated attempts at amniocentesis were necessary in 10 patients. Five repeat attempts were made because no fluid was obtained. In 7 cases there was failure of cell cultivation, of which in two cases a repeat attempt was not made. One patient had the pregnancy terminated privately and in the other the procedure was done specifically to exclude an open neural tube defect. There were dry taps in 5 (6.3%) and failure in cell cultivation in 7 (9.3%) cases.

Ultrasonic Scan in Midtrimester Amniocentesis

In 21 cases there was no scan done prior to amniocentesis, 13 were cases performed before ultrasound was available at the hospital, and a further 8 where no apparent reason was given. Of the 21 cases without a scan, one patient required two taps, and another had bloodstained fluid aspirated, hence multiple taps were necessary in one (0.5%) and a blood-stained fluid obtained in one (0.5%). Whereas in 54 cases who had a scan either before or simultaneously with amniocentesis, 8 (14.8%) required multiple attempts, in 4 (7.4%) because of a dry tap and in 4 because of a failure in cell cultivation. In two (3.7%) of the cases with scan done the fluid was blood-stained.

Alphafetoprotein Estimation for Detection of Open Neural Tube Defect

Alphafetoprotein estimation was done on 63 samples of amniotic fluid. Of the 12 samples not

assayed, 11 were collected before the value of this investigation was established. In the remaining case the result was not recorded and the pregnancy terminated for Trisomy 21. The levels of alphafetoprotein are shown on the plot below, (Figure I) and this shows that all the determined values fell below mean + 2 standard deviations, except one (Case 14) where the level was 34 ug/ml. at 21 weeks gestation. However, a normal male infant was delivered of this pregnancy, and the calculated period of gestation was in serious doubt. The fetus had no evidence of neural tube defect in 49 cases who had delivered or had termination of pregnancy performed. The remaining 13 cases were undelivered at the time of writing.

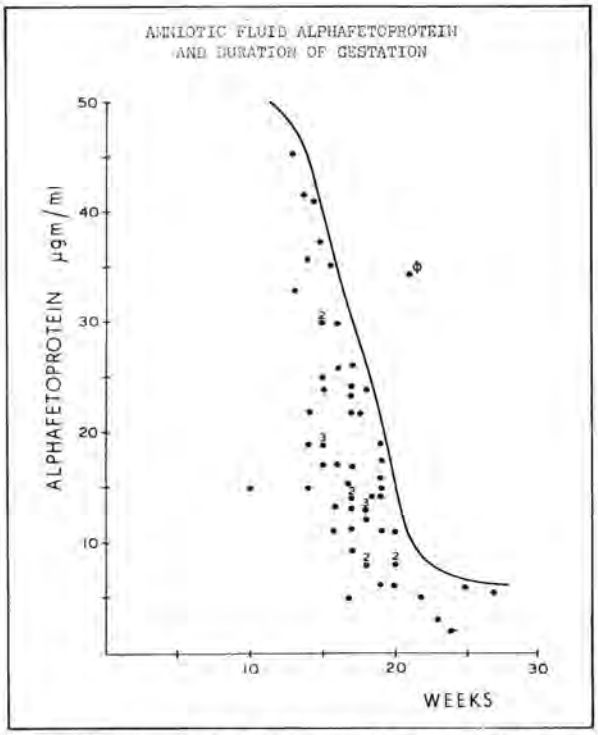

Fig. 1. Alphafetoprotein levels in amniotic fluid in 63 cases. Each symbol represents one case unless indicated by the numeral above it. The solid line represents the upper normal limit for alphafetoprotein in amniotic fluid (mean + 2 Standard Deviations).

1 Indicates Case 14, level of Alphafetoprotein, 34 /ml. However, the calculated gestation of 21 weeks was in doubt and she delivered a normal male infant.

Abortion Related to Amniocentesis

There was only one spontaneous abortion almost certainly related to the amniocentesis. This was a patient aged 36 years who had maternal age as the indication for the procedure. The patient started to lose amniotic fluid per vaginam the day after the procedure and this continued, in spite of bed rest in hospital. She aborted within a week of the procedure. This gives a risk of causally related abortion of at least 1/75 (1.3%).

Rhesus Negative Mothers Undergoing Amniocentesis

There were ten cases whose blood group was Rhesus D Negative. In five of these patients case notes, there was a specific mention made to the effect that Anti D Gammaglobulin had been administered after the procedure, in four no such mention was made, and in the other case there were already Rhesus D antibodies present.

Other Risks Associated with Amniocentesis

There were no cases of infection in this group, and no obvious injury (excepting the procedure-related abortion) sustained by the fetuses subjected to the procedure, though blood-stained fluid may be considered a feature of trauma to fetal vessels or fetal torso, and was noted in three cases, but since the Kleihauer test was not done on the red cells, maternal bleeding would be a possible cause of blood-staining of the fluid.

DISCUSSION

Antenatal diagnosis has become a practical procedure, and the area is rapidly evolving so that whereas the major indications were maternal age, previous Down's Syndome affected child, inborn errors of metabolism and X-linked recessive disorders, detection of neural tube defects is gaining in importance. Abnormal haemoglobin types and thalassaemias are the recent additions to conditions that can be diagnosed antenatally. A number of problems, however, are associated with midtrimester amniocentesis and diagnosis of chromosomal disorders and neural tube defects. Midtrimester amniocentesis is also not entirely free of risks and the problems, difficulties and precautions to be anticipated are as follows.

Problems in Antenatal Chromosomal Analysis

Three abnormal fetuses were detected out of 75

screened, a pick-up rate of 4% which is a reasonable discrimination in selection of patients (Scott, 1976). All these patients were above 35 years of age and older women are known to have a greater risk of having a child affected by Down's Syndrome than younger women. The risk to pregnant women 40 years and above is estimated to be about 2.6% and to women between 35-39 years of about 1.6% (Littlefield, 1974). Hence it is particularly important to screen women of advanced age of 40 years and above.

Anomalous karyotypes may be reported as in the three cases described, but in the first, a phenotypically normal male infant was delivered. Polypoidy is a significant problem in the interpretation of cytogenetic studies of cultivated amniotic fluid cells. Tetraploidy has been detected frequently in amniotic fluid cell cultures: 4% to 100% of cells (Milunsky *et al.*, 1971). In most cases the pregnancy has not been terminated and the infants born have had normal karyotypes. Possible explanations include an artifact of tissue culture or culture of cells derived from the amnion where tetraploidy occurs naturally. In the second case a phenotypically normal female infant was delivered whereas the result suggested that chromosomal mosaicism was present in the fetus. However, demonstration of mosaicism in a given individual may require study of more than one tissue. The finding of mosaicism in amniotic fluid cell cultures poses significant problems in terms of interpretation, for even if this finding was assumed to represent true mosaicism in the fetus it is often impossible to relate this to the phenotype of the child. There have been no documented reports so far of chromosomal mosaicism detected antenatally. In the third case the anomalous karyotype was due to maternal blood contamination, a normal male infant being delivered. A twin pregnancy was excluded by ultrasound scan. Nadler (1972) estimated that maternal cells would be growing on 0.5% of amniotic fluid cell cultures and this would lead to a number of false diagnoses. Phillip *et al.* (1974) reported two false sex predictions in 93 cases and they advocate the comparative study of Q-banded maternal and fetal chromosomes, since in most cases differences in fluorescent markers might enable a distinction to be made between mother and fetus. Mosaicism was considered highly unlikely for the reasons mentioned earlier. Regarding false sex predictions, therefore, there was a minimal error of 1/57 (1.8%) whereas Phillip *et al.* (1974) reported 2.2% in 93 cases.

Failures in Amniocentesis and Cell Cultivation

In this series there were repeated attempts in 6.3% whereas Niermeijer (1976) reported an incidence of 4% and Milunsky and Atkins (1974) in 10%. There was a 9.3% failure of cell cultivation whereas it was 1% and 10% respectively in the aforementioned series. Of the 7 cases of failure in cell cultivation, two were probably due to delay or contamination of the sample. In five cases there was no apparent reason and so a 6.3% incidence could be expected even with special care exercised to prevent delay and contamination before the sample reached the laboratory. This is in keeping with other reported series.

The length of time taken to obtain the karyotype following the dispatch of the sample to the genetic laboratory was on the average 4 weeks (Carter, 1978, Personal communication). This is an important criterion in evaluating antenatal diagnosis so that termination of pregnancy could be performed before fetal movements are felt (Ferguson Smith, 1971 and Golbus *et al.*, 1974).

Value of Ultraosonic Scan

The value of ultrasonic scan in midtrimester amniocentesis is the ability to localize the placenta and avoid trauma. Though its value is at present generally accepted, Gerbie and Vikolnik (1975) found the percentage of bloody taps to be the same whether a scan was done or omitted. Harrison *et al.* (1975), however, did note a decrease in blood-stained fluid with ultrasonic placental localization as did Crandon (1978, Personal communication). Though complications of midtrimester amniocentesis are few it seems logical to try and decrease even this small risk further by use of ultrasonic placental localization.

Alphafetoprotein Estimation for Detection of Open Neural Tube Defect

There was one case where the alphafetoprotein was falsely elevated due to an error in the calculated gestation as the date of the last menstrual period was in doubt. However, both false positive and false negative values are known to occur and Milunsky and Alpert (1976) reported their

experience of a 4.9% false positive rate. There were no false negative values in this group of cases. Accurate documentation of gestation is critical in interpretation of the result of alphafetoprotein.

Risks of Amniocentesis

The one causally related abortion following amniocentesis gives a risk of 1.3% in this series whereas it was 0.65% in Nadler and Gerbie (1970) and 1.47% in Milunsky and Atkins (1974) series. This is not significantly different from the risk of spontaneous abortion in the second trimester of approximately 1.2%.

No other fetal trauma resulted in this series whereas Lamb (1975) reported a case of fetal limb gangrene from needle puncture in midtrimester amniocentesis. The rhesus negative mother should have Kleihauer test done soon after the procedure and an extent of feto–maternal haemorrhage gauged and anti-D gammaglobulin given to those at risk.

CONCLUSION

In conclusion, antenatal diagnosis by midtrimester amniocentesis has been found to be a safe though not entirely risk-free procedure in this study as well as in a controlled study (Milunsky, 1975) and, when large numbers of cases are studied (Galjaard, 1976) and therefore it should be used more widely in appropriate cases for the benefit of the patient and perhaps her unborn child.

SUMMARY

Amniocentesis was performed in 75 patients for antenatal diagnosis of chromosomal abnormalities and neural tube defects. Three fetuses with Trisomy 21 were detected, and the pregnancy terminated. In three other cases there was an anomalous karyotype reported, in which all were delivered of phenotypically normal infants. There was one case where the sex prediction was in error. No case of neural tube defect was detected by alphafetoprotein estimation, whereas one case had a false positive value due to uncertain gestation. There was one causally related abortion. The use of ultrasonic placental localization was not associated with a decressed incidence of need for repeat attempts or blood-stained fluid. The value, risks, difficulties and precautions for midtrimester amniocentesis are discussed.

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