PRENATAL CYTOGENETIC DIAGNOSIS IN 150 MID TRIMESTER AMNIOCENTESES

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SUMMARY

Cytogenetic results of 150 amniocentesis performed during second trimester are presented. The indications for the amniocentesis were advanced maternal age, previous child with chromosome disorder, parental balanced translocation, congenital abnormality in the previous fetus, abnormal fetal sonogram, repeated fetal loss and mosaicism observed in CVS cases. Success rate for culture was 96.7%. The average reporting time was between 12-16 days, the minimum time being 8 days and maximum time was 28 days. 6 analysis were found to be abnormal, out of which 5 pregnancies were terminated and 1 patient was lost for follow up. Two patients who had single cell mosaicism in amniotic fluid culture were subjected for fetal blood sampling. The analysis of which was found to be normal. Study included 5 twin pregnancies.

INTRODUCTION

Prenatal diagnosis using amniotic fluid cells was established when Steel & Breg (1966) demonstrated viable fetal cells in amniotic fluid and their ability to yield mitosis on culture. Since then chromosome analysis of the fetus using amniotic fluid cells during the second trimester is offered in many countries all over the world. The chromosomes thus obtained reflect the fetal karyotype. Various types of cells from diverse origin like fetal skin & urine and amniotic sac are present in the amniotic fluid. Martin (1980) has given detailed classi-

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fication of these cells from morphological appearance. However, we have observed 3 main types of cells (i) Round cells (ii) Polygonal cells and (iii) Fibroblast type of cells. Present paper is based on the study of 150 samples studied during 16-20 weeks of gestation. Indications for the fetal karyotype were :

(i) Advanced maternal age.
(ii) Previous child with Down's Syndrome, trisomy 21.
(iii) Previous child with trisomy 13/18.
(iv) Parent translocation carrier.
(v) Maternal serum low α fetoprotein levels.
(vi) Congenital abnormality in the fetus.
(vii) Abnormal fetal sonogram.
(viii) Repeated fetal loss.

The patients were counseled regarding the procedure of collection of sample, the risk of miscarriage involved, reliability of the test and the necessity for fetal blood sampling in case the abnormal karyotype is found in the form of mosaicism.

MATERIAL AND METHODS

Total of 150 samples were studied. Approximately 20 ml of clear amniotic fluid was collected in a good quality 20 ml disposal syringe under ultrasound guidence in the operation theatre by an obstetrician. The samples were transfered in a transport vial and reached to the laboratory immediately. For initial 50 samples, the blood stained samples were not set up for culture. Later on the cultures were also set up for blood stained samples. Each sample was divided into 4 parts and the fluid was centrifuge at 1000 RPMI for 10 minutes. The supernatent was removed leaving 0.5ml in the centrifuge tube. Small quantity of supernatent fluid was sent for α fetoprotein estimation wherever necessary. To the pallet in 3 different centrifuge tubes was added 5 ml of Ham F-10 medium supplimented with 20% Fetal bovine serum and one drop of antibiotics in the form of peniciline and streptomycin. In the fourth centrifuge tube the Chang medium was used. Fluid was then set up for culture in four different tissue culture flasks in 5% CO_2 atmosphere. The open culture system was used. The flasks were left undisturbed for 8 days. After this period the growth of the cells was observed under inverted microscope. Further, on every alternate day complete medium change was performed till sufficient mitosis were observed. Usually sufficient mitosis were observed from 12 days to 15 days. At this stage the cultures were harvested as per the standard protocol. The slides were further allowed to dry for 2 days and then subjected for 'G' banding and 'Q' banding techniques and observed in the high resolution microscope. At least two flasks were harvested and total of 30 metaphases were studied for each flask. Whenever abnormal karyotype was found in mosaic pattern further flask also was harvested. The metaphases were photographed and karyotype prepared.

RESULTS

Results of the fetal chromosome analysis were available between 8 days to 23 days, average time being 13 days. Table I shows the number of cases along with the indications. There was no immediate fetal loss after the procedure

and no serious complication to the mother. Out of 150 pregnancies 140 are delivered and 10 pregnancies are on going. Out of 150 samples, successful cultures were obtained in 140 cases, 5 samples could not yield results because of bacterial contamination or blood in the samples. Out of 145 successful cultures 6 were abnormal - 2 were trisomy 21, out of which 1 free trisomy 21 (Down's Syndrome) to an elderly mother (42 yrs.) and 1 due to (21/21) maternal in origin, 1 fetus had 46, XX, 18p- chromosome analysis, 2 patients had presence of marker chromosome and 1 patient had single cell mosaic trisomy 2. Five pregnancies were terminated and for the last patient, fetal blood sampling was recommended but the patient was lost for follow up. Table II shows the pregnancies involving balanced translocation in the parents along with the pedigree and involved chromosome. 8 analysis were normal and 1 was trisomy 21. Chromosome analysis of the parents was done prior to amniocentesis because of bad obstetric history in the form of birth of an abnormal child or early miscarriages.

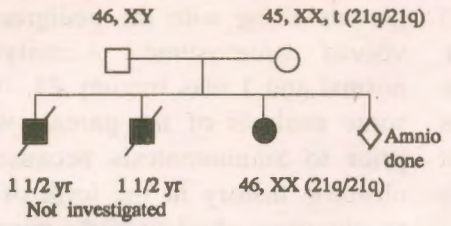
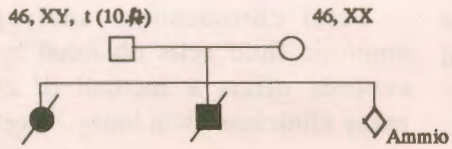
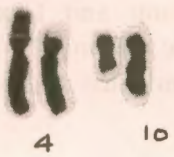
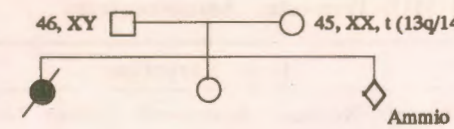
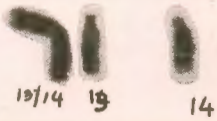
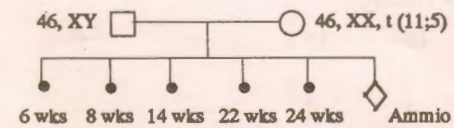
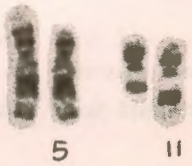
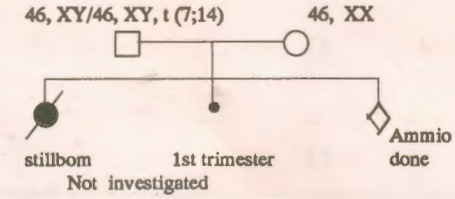
DISCUSSION

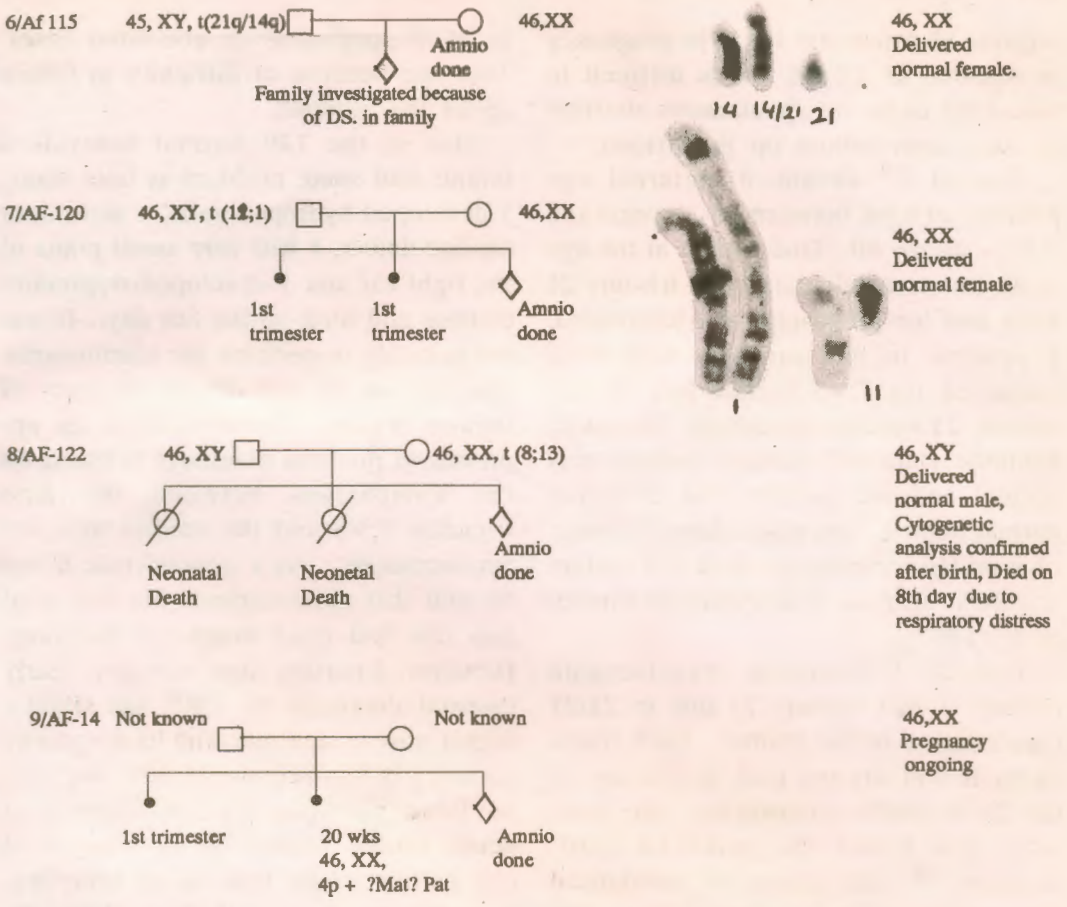
Fetal chromosome analysis using amniotic fluid cells obtained by amniocentesis offers a method of choice to many clinicians even today. Even though

Table I

Cytogenetic Results of 150 MID-Trimester Amniocenteses

Sr. No.	Indication	Fetal Karyotype				
		No. of cases	Normal	Abnormal	Carrier	No Results
1.	Advanced Maternal Age	57 (4 twins)	55	2	—	4
2.	Previous child with Down's Syndrome, Trisomy 21	18	18	—	—	—
3.	Previous child with Trisomy 18/13	4	4	—	—	—
4.	Parent translocation carrier	9	8	1	—	—
5.	Altered maternal serum α fetoprotein levels	4	4	—	—	—
6.	Congenital abnormality in the fetus	41	37	3	—	1
7.	Abnormal fetal sonogram	4	4	—	—	—
8.	Repeated fetal loss	13	13	—	—	—
		150	143	6	—	5

Sr. No. / Lab. No.	Pedigree	Reciprocal balanced/ Robertsonian translocation in parents	Results & out come
1/AF-15			46, XY,t (21q/21q) pregnancy terminated
2/AF-43			46, XX Delivered normal female
3/AF-87			46, XX Delivered normal female.
4/AF-90			46, XX delivered normal female.
5/AF-100			46, XX Delivered normal female.



first trimester fetal diagnosis is possible by chorion villous sampling, the facilities for the same are not always available. The specimen needs to be transported to the laboratory very quickly after collection. MRC working party in 1991 has reported 4.6% reduction in successful pregnancy outcome in first trimester CVS as compared to second trimester amniocentesis. A large Canadian trial in 1980 has reported slight increase in the risk of unsuccessful pregnancy outcome in CVS as compared to second trimester amniocentesis. In view of these observations amniocentesis is useful for high risk and precious pregnancies.

Kappel et al (1987) found the spontaneous abortion rate following mid-trimester amniocentesis to be 2%. United States of National Amniocentesis Registree (NICHD-1976) reported 3.5% fetal loss rate. The report of Medical Research Council (1978) noted spontaneous abortion rate as 2.5%. In the present 150 analysis 2 patients aborted within 1 week and 1 patient had profound amniotic leak immediately after procedure. However, this patient settled down after appropriate treatment. We had 4 twin pregnancies in the present study. Spon-

taneous abortion rate for twin pregnancy is reported as 12.5%. It is difficult to assess the cause for spontaneous abortion as long term follow up is difficult.

Out of 57 advanced maternal age patients 49 were between 35-39 years and 8 were above 40. One patient at the age of 42 had prenataly diagnosed trisomy 21 fetus and the pregnancy was terminated. 2 patients in this category who were subjected for CVS earlier had mosaic trisomy 21 and 20 respectively. However, amniotic fluid cell culture analysis was normal and the patients had delivered normal babies. The discordance between chorion tissue, amniotic fluid cell culture and the fetal tissue is discussed by Simoni et al (1983).

Out of 9 balanced translocation patients 1 had trisomy 21 due to 21/21 translocation in the mother. Such translocation will always lead to trisomy of the 21 in viable pregnancies. No other fetus had either the balanced chromosome like the parent or unbalanced translocation. Their pedigree shows that all of them had bad obstetric history in the form of first trimester miscarriages or fetal wastage at a later stage. The success rate for culture was 96.7% in the present study. Failure to get the results of the remaining samples was due to fungal or bacterial contamination at the source and/or in the laboratory, heavily blood stained samples and on one occasion very poor response of the cells for the growth. This patient was then subjected to fetal blood sampling. Diagnostic accuracy was good. However, it was difficult to perform the confirmatory karyotyping in normal babies or in MTP specimens in abnormal cases. This was because of difficulty in follow up of the patients.

Out of the 149 normal analysis 4 infants had some problem at later stage, 1 developed hydrops fetalis, 1 had minor cardiac defect, 1 had very small pinna of the right ear and 1 developed respiratory distress and died on the 8th day. It was not possible to perform the chromosome analysis on all the infants because of various reasons. In conclusion, an approach to prenatal diagnosis is based on the comparison between the first trimester CVS and the second trimester amniocentesis. As a general rule it can be said that amniocentesis has less fetal loss rate and good diagnostic accuracy. However, reporting time is longer. Early prenatal diagnosis by CVS has slightly higher miscarriage rate and its diagnostic accuracy is less because of false negative or false positive reports. Hence, if needs further testing by amniotic fluid cell culture or by fetal blood sampling. However, termination if required is early if abnormal karyotype is detected. The general policy observed was to explain to the patient both the procedures and then perform the test. However, early aborter group of patients and patients with the balanced translocation were always subjected for amniocentesis because chromosomes obtained were of very good quality for detail banding.

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