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Cytogenetic Analysis from Mid-Trimester Amniotic Fluid Culture: Technical Aspect

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ABSTRACT

Social problems and economical burden of the children born with chromosomal abnormalities is tremendous. Primary prevention by prenatal diagnosis appears to be a better mean to deal with the problem rather than expensive treatment. But diagnostic procedures are also costly due to equipments and reagents required. So this study aims to analyze midtrimester amniotic fluid samples and to find out difference between the results with and without using CO2 to make it cost effective. 50 Amniotic fluid samples were obtained from high risk pregnancy group. Cultures were obtained by standard protocol. Adequate culture growth was observed in all 50 samples. No significant difference was observed in the number of days required for the culture to grow with or without using CO2. To conclude amniotic fluid cultures can be grown adequately even without using CO2, this makes the technique cost effective and simpler for successive management of the cases.

Keywords: Karyotype, Amniotic fluid culture, chromosomal aberrations, CO2 incubator.

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INTRODUCTION

Social problems and economic burden to the families and society associated with the population of children born with chromosomal abnormalities is tremendous [1]. These abnormalities can be detected by prenatal diagnosis which involves coordinated efforts from different disciplines of medicines for example Geneticist, Obstetrics and Gynecologist, Pediatrician, Radiologist etc.

It is observed that chromosomal aberration occurs in at least 0.6% of live born children and 2/3rd of these are associated with substantial disability [2]. Primary prevention appears to be a better mean to deal with the problem rather than treating the handicaps by Gene therapy or by other means which are very expensive and therefore not affordable for people from all socio-economical class. Primary prevention can be achieved by prenatal genetic diagnosis and could be for single gene, chromosome or multifactorial inheritance disorder. Prenatal chromosome analysis aims to rule out any nondisjunction or structural aberration in the diploid set of chromosomes and thus gives confidence for continuation or option for termination of pregnancy.

Chromosomal analysis of foetus can be performed at –

- 11-12 weeks of gestation by Chorionic villus sampling
- 16-20 weeks of gestation by Amniocentesis
- 20 weeks onwards by Foetal blood sampling

All these procedures are done under ultrasonographic guidance. Amniocentesis involves drawing of amniotic fluid from amniotic cavity for chromosomal analysis. Amniotic cavity appears as a small space between the cytotrophoblast and the embryoblast cells during 2nd week of gestation. Its roof is lined by amniogenic cells derived from Trophoblast while the floor is ectodermal in origin. Amniotic cavity is filled with amniotic fluid and it completely surrounds the embryo as fluid level gradually increases with advancing gestational age [3-5].

Amniocentesis is done in 2nd trimester between 16-20 weeks of gestation. It is observed that the laboratory cost for amniotic fluid culture and analysis are high. It is because of the use of expensive culture media, culture vessels (flasks, tubes) and laboratory equipments like CO2 incubator and also trained personnel.

Total time required for culture and analysis are usually more than 10 days. Newer techniques like FISH (Fluorescent in Situ Hybridization) can provide diagnosis for some numerical chromosomal anomalies within 2 days. However FISH findings need to be supported by gold standard karyotyping by culture.

This study aims to process amniotic fluid by 2 methods and laboratory techniques. Half of the sample is cultured with the use of CO2 incubator (5%CO2) and half of the sample without CO2 incubator. Following parameters are considered

- Quantity & quality of cell growth in cultures by both methods.

- Time required by both techniques.
- Cost involved in each technique.

The technique without using CO2 incubator will be suitable for cytogenetic tests at district level and the need for samples to be transported to bigger cities will be eliminated. It will also reduce the cost for patients to come to bigger cities as an alternative for sending samples.

Aims

To analyze midtrimester amniotic fluid samples and find out the difference between results with and without using CO2.

Objectives

- To do amniotic fluid culture with and without using CO2.
- To find out difference between the two methods.
- Culture growth
- Cost effectiveness
- Time period

MATERIAL AND METHODS

Sample size: 50

3ml- 5 ml of amniotic fluid was obtained in sterile container from the samples received by Authorized laboratory. No separate amniocentesis was performed for the study purpose and Ethical Committee clearance was taken mentioning the same.

Clinical history of the patient was recorded.

- Age of the patient
- Last Menstrual Period (1st day of the last cycle)
- Duration of pregnancy in weeks
- Obestric history
- Reason for reference

Physical parameters were noted:

- Quantity/ Volume (ml)
- Color- clear/ slightly blood stain/ heavily blood stained/ discolored (brown)

Culture was obtained by standard protocol which included:

- Planting
- Harvesting
- Banding

- Screening

Chromosomes were studied by scanning slides first under **10X** and then **100X.**

Data obtained was analyzed statistically and results obtained were discussed.

RESULTS

Cytogenetic analysis of 50 amniotic fluid cultures was performed. All the samples were obtained from high risk pregnancy group from authorized laboratories after taking informed and written consent. Indication of cases selected for study was advanced maternal age, high risk triple marker, and abnormality detected on ultrasonography. All the samples were cultured with and without using CO2.

Adequate culture growth was observed in all 50 samples. It was observed that as the gestational age advances the number of days required for the culture to grow adequately increases. It took only 9-11 days when the gestational age was 16-18 weeks whereas as the gestational age advanced to 20 weeks culture took as long as 14 days to grow (Table no: 1) . As depicted in table no 2, no significant difference was observed in the number of days required for the culture to grow with or without the use of CO2. Almost 19 samples took same number of days with and without using CO2 and even in other samples the difference was not more than a day. It was also found that the test becomes comparatively cheaper without the use of CO2 and also made the methodology and technique simpler. It was observed that cell growth in culture with or without CO2 was irrespective of maternal age and blood contamination of the samples.

Table 1: Shows comparison between adequate cell growths obtained with or without using CO2 and the gestational age of the patients.

Sr.no	Pregnancy in weeks	No. of patients	Adequate cell growth in days	
			With CO2	Without CO2
1	16 wks	17	9-10 days	9-10 days
2	17 wks	13	9-11 days	9-11 days
3	18 wks	6	11-12 days	11-12 days
4	19 wks	7	12-14 days	12-14 days
5	20 wks	7	13-14 days	13-14 days

Table 2: Shows comparison between the numbers of days required by the samples to grow adequately by each method

No. of samples with faster growth in CO2	No. of samples show faster growth Without CO2	No. of samples with equal growth in both
16	15	19

Out of the 50 cases studied 47 cases (94%) were advanced maternal age, 38 cases (76%) showed high risk triple marker test and 3 cases (6%) showed abnormal ultrasonography. It was also seen that out of 3 cases with abnormal ultrasonography

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findings, 1 was found to have Trisomy 18 (47, +18) suggesting the need of prenatal diagnosis in cases of abnormal ultrasonographic marker (Table no: 5).

Karyotype result of all the samples were compared with the different age group of patients. All the samples show 46 normal chromosome complements except one in the 41-45 years age group which showed (47, +18) trisomy 18. Thus it suggests increased susceptibility to chromosomal abnormality as maternal age advances (Table no: 3 & 4).

Table 3: Shows comparison between different age groups of patients and karyotype results.

Sr.no	Age Group	No. of patients	Adequate cell growth in days		Result
			With CO2	Without CO2	
1	31-35 yrs	3	9-14 days	9-14 days	Normal 46 chromosome compliment
2	35-40 yrs	39	9-14 days	9-14 days	Normal 46 chromosome compliment
3	41-45 yrs	8	9-14 days	9-14 days	All show 46 N chromosome except One case with 47,+18

Table 4: Shows cases with advance maternal age, abnormal triple marker and abnormal ultrasonography in the study.

Sr.no	Reference reason	No. of patients	Adequate cell growth in days		Result
			With CO2	Without CO2	
1	Advance maternal age	47 (94%)	9-14 days	9-14 days	All the samples show 46 normal chromosome compliments except One case with 47,+18
2	Abnormal triple marker	38(76%)	9-14 days	9-14 days	
3	Abnormal USG	3(6%)	9-14 days	9-14 days	

Table 5: Shows the cases with abnormal ultrasonography finding.

Sr.no	Abnormality seen on USG	No & age of patients	Adequate cell growth in days		Result
			With CO2	Without CO2	
1	Cisterna magna	1- 42 yrs	10 days	10 days	Normal 46 chromosome compliment
2	Hydrocephalus	1- 35 yrs	11 days	11 days	Normal 46 chromosome compliment
3	Cerebro-pontine cyst+ VSD	1- 41 yrs	14 days	13 days	47,+18 (Edward syndrome)

DISCUSSION

Prenatal diagnosis plays an extremely important role in genetic counselling. Over a period of time there has been a rapid development in the field of prenatal diagnosis. Many

new methodologies and techniques have been introduced in prospective of getting rapid and better results.

Since introduction of cytogenetic analysis from Amniotic fluid cells this method has become a standard test for antenatal diagnosis according Boronova et al 2006 [6]. New methods in prenatal diagnosis like rapid aneuploidy test, Fluorescent Insitu Hybridization allow demonstrating certain numerical aneuploidies in amniotic fluid cells in almost 24 hours as compared to conventional method which takes about 1-3 weeks.

According to Dudarewitz et al 2005 rapid aneuploidy tests cannot be used as stand-alone test for prenatal diagnosis but are used as preliminary test till the cytogenetic karyotyping is ready. A karyotype using classical banding should be performed in every case with whatever indication [7]. It is the only method which helps in detection of all chromosome abnormalities and so serves as gold standard test according to Boronova et al. 2006 [6].

Kucheria et al 2002 suggested that Up to 95% of live born cytogenetic abnormalities are because of chromosomal aneuploidy like trisomies 13, 18, 21, monosomy X and 47 XXY [8].

In the present study (Table no: 4 & 5) 50 patients were selected on the basis of referral reasons. When there referral reasons were evaluated it was seen that 94% 0f cases were advanced maternal age, 76% had high risk triple marker test, 64% had both and 6% cases showed abnormal sonography findings.

Sung Hee Han et al, 2008 in their study on 31,615 cases observed distribution of patients according to the indication as 18.4% cases with advanced maternal age, 1.3% cases with previous chromosomal anomaly and 1.1% cases with previous congenital anomaly, 0.6% with family history of chromosomal abnormalities, 0.2% cases with congenital anomaly, 0.1% were carrier of X linked disorder, advanced maternal screening 69.5%, abnormal ultrasound 5.7%, stillbirth 1.2%, maternal anxiety 1.1% and 0.8% with twin pregnancy. Thus abnormal maternal serum screening was found to be the most common indication followed by advanced maternal age [9].

In our study cultures for most of the samples showed adequate growth by 9 to 12 days (Table no: 3 & 4). However it was seen that cultures for samples with gestational age beyond 18-19 weeks took almost 14 days to grow adequately though there was no case of cell culture failure because of increased gestational age or in case of blood stained samples. Lam YH et al 1998 mentioned about more culture failure rate (10%) in cases where samples were obtained at 24 week of gestation as compared to samples obtained before 24 weeks (0.27%) of gestation [10]

Boronova et al, 2006 found 80 to 100% success rate in obtaining culture, whereas overall success rate for obtaining cytogenetic diagnosis was found to be 91.7%.They also mentioned about absence of cell culture growth in late gestation samples and in blood contaminated samples [6]. Zahed L et al 2000, mentioned overall 0.9% culture failure rate in their study, maternal cell contamination was observed in 0.6% [11].

Pregnancies with Amniotic culture failure and also mentioned high prevalence of abnormalities on Ultrasonography in culture failure cases as compared to those with adequate culture growth because of decrease fetal cell shedding [12].

In present study, no significant difference was observed in the number of days required for the culture to grow with or without use of CO2. It was found that the test becomes comparatively cheaper without the use of CO2. This also makes the methodology and technique simpler and affordable for implementation.

In the present study all the samples except one showed 46 normal chromosome complements. Only one case of (47, +18) Trisomy 18 was observed in the 41-45 years age group (Table no: 4). Thus it suggests increased susceptibility to chromosomal abnormality as maternal age advances. Significant association of maternal age with aneuploidy and miscarriage is recognized since long and maternal age factor is demonstrated in varying magnitude in most of the trisomies [12-15]. Ferguson-Smith et al 1971observed that out of 115 mothers in age group above 40 years 5 fetuses were found to have chromosomal abnormality amongst which 4 fetuses had down`s syndrome and one had Klinefilter`s syndrome [16].

Sung Hee Han et al, 2008 found 3.1% cases with chromosomal abnormality out of the 31,615 cases studied. In similar study conducted by karaoguz et al and Tseng et al these percentages were found to be 3.0% and 2.9% respectively [9]. Down syndrome was most common abnormality 36.9%. Out of these classical down were 88.9%, mosaicism 2.5%, Robertsonian translocation 8.6% cases. They also found increased prevalence of down since 1994, may be due to advanced maternal age. Out of the sex chromosomal abnormalities Turner syndrome was most common (0.2%).

Boronova et al analyzed 370 samples by G-banding and normal karyotype was observed in 356 (96.2%) cases and 14 samples (3.8%) were found to have abnormal karyotype. Out of these Generalized mosaicism was found in 3 samples (0.8%), pseudomosaicism was detected in 2 (0.5%) cases, Numerical abnormality was seen in 3 cases (0.8%). 11 cases (3%) were found to have structural aberration. Pericentric inversion of chromosome number 9 was the most common abnormality found in 6 cases (1.6%) whereas mosaic karyotype was seen in 3 cases (0.8%) [6].

In the present study it was observed that out of 3 cases with abnormal ultrasonography findings (Table no: 5), 1 was found to have Trisomy 18 (47, +18) suggesting the need of prenatal diagnosis in cases of abnormal ultrasonography findings. In their studies Yang et al, Tseng et al and Karaoguz et al mentioned highest detection rate of chromosomal abnormalities in prenatal diagnosis by abnormal ultrasonography finding as 6.5%, 8.9% and 5.3% respectively [9]. Sung Hee Han et al, also found abnormal ultrasonography rate (5.9%) as highest positive predictor of chromosomal abnormalities in their study [9]. Campbell S et al 1983 also suggested significance of ultrasonography in detection of various congenital malformations [17]. This suggests high sensitivity of ultrasonography in detection of anomalies which intern necessitate amniocentesis for cytogenetic analysis.

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CONCLUSION

Cytogenetic analysis from midtrimester amniotic fluid culture is gold standard test for detection of almost all chromosomal anomalies. Cultures can be grown adequately even without using CO2. Culture without CO2 where the screw cap is tightly closed helps in prevention of cross contamination and thus the need for repeat test. This makes the technique cost effective and simpler for implementation even at district level. So the need of transferring the sample to big cities will be reduced and delay in delivering results will be prevented. This will eventually help in successful management of patient and reduce burden of chromosomal abnormalities in patients.

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