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Role of Multicolor Fluorescence in situ Hybridization (FISH) in Simultaneous Detection of Probe Sets for Chromosome 18, X and Y in Uncultured Amniotic Fluid Cells

Major aneuploidies diagnosed prenatally involve the autosomes 13, 18, and 21, and sex chromosomes. Fluorescence in situ hybridization (FISH) allows rapid analysis of chromosome copy number in interphase cells. The purpose of this study was to evaluate the role of multicolor fluorescence in situ hybridization in simultaneous detection of probe sets for chromosome 18, X, and Y in uncultured amniotic fluid cells as a safer alternative method for aneuploidy detection prenatally. Fifty amniotic fluid samples were analyzed by FISH and standard cytogenetics. Mean time to obtain results was three days for fluorescence in situ hybridization and 20 days for karyotype. Fluorescence in situ hybridization was informative in 43 samples (86%), and within this group, two aneuploidies were correctly identified. This evaluation demonstrates that FISH with X, Y, and 18 alpha satellite DNA probes could accurately and rapidly detect aneuploidies involving these chromosomes and could be used in any prenatal clinical laboratory.

Key Words: *Prenatal diagnosis; In situ hybridization, fluorescence; Amniotic fluid*

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INTRODUCTION

The majority of aneuploidies diagnosed prenatally involve the autosomes 13, 18, and 21, and sex chromosomes. Aneuploidies involving these five chromosomes can count for up to 95% of all liveborn chromosomal abnormalities which are accompanied by birth defects (1). The ability to analyze the fetal karyotype is an important issue in prenatal diagnosis. However, conventional cytogenetic analysis of amniocyte is limited because it is labor intensive, time consuming, and require a highly trained analysis. Thus, there seems to be a need for rapid methods for detecting major abnormalities. New methods such as fluorescence in situ hybridization (FISH) allow the detection of chromosome aberrations using chromosome-specific probes. Fluorescence in situ hybridization (FISH) on uncultured amniocytes with use of chromosome-specific probes has been described for the rapid diagnosis of numeric abnormalities affecting chromosomes 13, 18, 21, X, and Y (2-5). FISH on uncultured amniocytes has the capability of diagnosing these aneuploidies within 24 to 48 hr of amniocentesis (6). Amniocentesis with FISH on uncultured amniocytes may be a safer alternative for aneuploidy detection compared to fetal blood sampling. But due to the lack of data regarding its general applicability in prenatal diagnosis, the clinical role of FISH with amniocentesis is still poorly defined. The recommendation is that it should only be used in conjunction with standard cytogenetic analysis (7). We used three DNA centromeric probes (X, Y, and 18 alpha satellite DNA probes) commercially available for prenatal diagnosis of chromosomal abnormalities in uncultured amniocytes by FISH. The purpose of this study was to evaluate the role of multicolor fluorescence in situ hybridization in simultaneous detection of probe sets for chromosome 18, X, and Y in uncultured amniotic fluid cells as a safer alternative method for aneuploidy detection prenatally.

MATERIALS AND METHODS

From October 1997 to June 1998, FISH on uncultured amniocytes has been offered at our institution as an adjunct to conventional chromosomal analysis to all patients having genetic amniocentesis who fulfilled certain indications between 18 and 24 weeks of pregnancy (Table 1).

All patients were informed of the investigational role

Table 1. Indications for FISH and karyotype analysis

Indication	No.	%
Abnormal triple screening	23	46
Advanced maternal age	19	38
Congenital anomaly	8	26
Total	50	100

Table 2. Reasons for uninformative or unreportable FISH results

Reason	No.
Blood stained amniotic fluid	3
Insufficient cell for analysis	2
Technical failure (FISH)	2
Total	7

of FISH in prenatal diagnosis. The results of the FISH analysis were considered uninformative if there was inconclusive hybridization pattern or technical difficulties in performing the analysis. FISH was not performed and results were considered unreportable if visible blood contamination was present (Table 2).

Cells

Fifty samples of 15-20 mL of amniotic fluid (AF) were obtained by amniocentesis between 18 and 24 weeks of pregnancy from patients of abnormal triple screening, advanced maternal age, and ultrasound fetal anomalies. Ten mL of AF was used for standard karyotyping. The remaining AF was used for direct in situ hybridization analysis.

Slide preparation

Uncultured amniotic fluid were centrifuged at 18,000 rpm for five min after direct adding 100 μL of Carnoys solution. Then resuspended pellet in 100-200 μL of fresh Carnoys solution, then centrifuged at 18,000 rpm for five min to collect cells. Place 50 μL of cell suspension on clean slide after centrifuge, and complete air dry.

DNA probes

We used DXZ1 (directed labeled in Spectrum Red), DYZ1 (directed labeled in Spectrum Green), and D18Z1 (directed labeled in Spectrum yellow) alpha satellite DNA probes corresponding respectively to chromosome X, Y, and 18.

Fluorescent in situ hybridization

Dehydrate slide with series of EtOH washes for two min each and air dry. The 10 μL probe mix of CEM buffer, 0.5 μL X (Vysis), 0.5 μL Y (Vysis), and 0.5 μL 18 (Vysis) probes. Place the probe mixture on slide and sealed with rubber cement and allow to complete air dry before transferring the slide to the flat bed of a HYBRID Omnigene thermal cycler for denaturation at 80℃ for one min, then allow the slide to hybridize for 30 min in humidity chamber at 37℃.

Place the slide in 0.5×SSC at 68℃ for three min. After three times washes in 1×PBD, the nuclei were counterstained with DAPI and reviewed using a Zeiss Axioskop with triple band pass filter that allows simultaneous visualization of green, red, and yellow signals on a blue background.

Cytogenetic analysis

From each sample, parallel cultures were grown in culture flasks with five mL of amniotic fluid plus five mL of Ham's supplemented with 20 percent fetal calf serum and one per cent Ultroser G. After five days, the cultures were inspected and the medium was changed twice a week. Primary cultures were stopped when at least five mitotically active colonies were visualized. The cells were harvested using trypsin-EDTA. Chromosomes were prepared according to standard cytogenetic methods. The karyotyping results were obtained using G- or R-banding techniques.

RESULTS

During the study period, fifty patients had amniotic fluid specimens sent for both FISH on uncultured cells and conventional karyotyping. The mean maternal age at amniocentesis was 29.7 years (range 17 to 39 years, SD 4.3), and the mean gestational age at amniocentesis was 20.6 weeks (range 18 to 24 weeks, SD 3.8). The mean time to obtain results was three days for FISH (range two to five days) compared with 21 days for conventional karyotype (range 19 to 28 days). FISH results were reported significantly faster than those of karyotyping. Time taken for results of either procedure did not vary by gestational age or by presence of fetal abnormality. There were no karyotype culture failures in our study. Over all, 43 (86%) FISH specimens were informative, with 7 (14%) being uninformative or unreportable. The reasons for an uninformative or unreportable FISH results are presented in Table 2. Nuclei from uncultured amniocytes were smaller, less intense, more patchy and condensed in Fig. 1. Analysis of 10-200 nu-

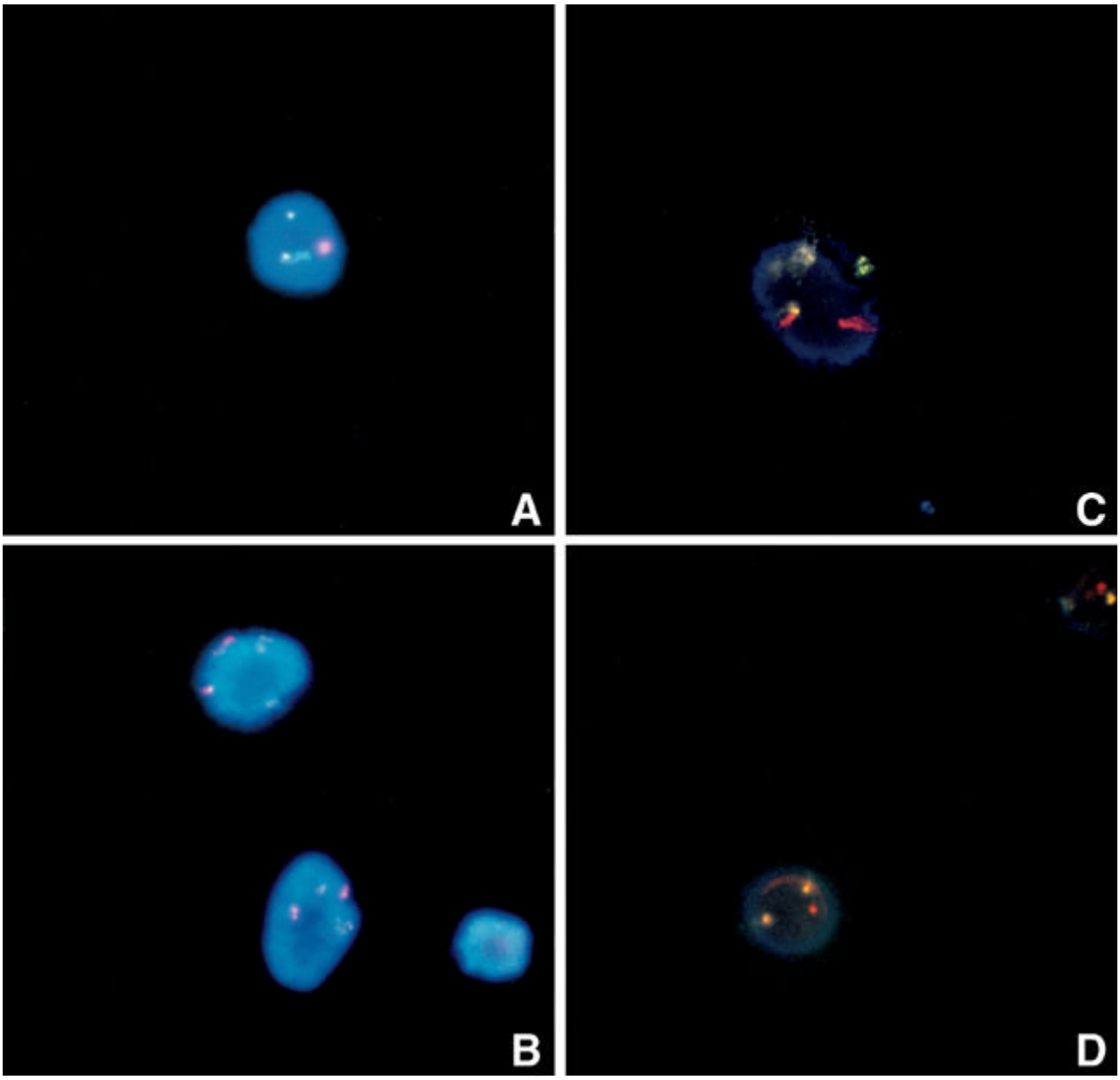

Fig. 1. **A:** Interphase nucleus from uncultured amniocytes by FISH shows one green (Y chromosome), one red (X chromosome), and two yellow (chromosome 18) signals. **B:** Interphase nucleus from uncultured amniocytes by FISH shows two red (X chromosome), and two yellow (chromosome 18) signals. **C:** Interphase nucleus from uncultured amniocytes by FISH shows two red (X chromosome), one green (Y chromosome), and two yellow (chromosome 18) signals. **D:** Interphase nucleus from uncultured amniocytes by FISH shows one red (X chromosome), and two yellow (chromosome 18) signals.

clei per patient and per probe was performed. The means and standard deviations of the percentage of hybridization domains with X, Y, and 18 probes observed in samples are indicated in Fig. 2. Analyses of sex chromosome and autosome 18 are summarized in Table 3. All the sex diagnoses and two sex aneuploidies predicted by FISH were confirmed by full karyotyping. Chromosome 18 copy number was correctly determined by FISH in all the samples.

DISCUSSION

We have presented that FISH provides efficient and accurate prenatal detection of chromosomal aneuploidies in uncultured cells from amniotic fluid. The majority of aneuploidies diagnosed prenatally involve autosomes 13, 18, and 21, and sex chromosomes. Aneuploidies involving these five chromosomes can count for up to 95% of all liveborn chromosomal abnormalities which are accom-

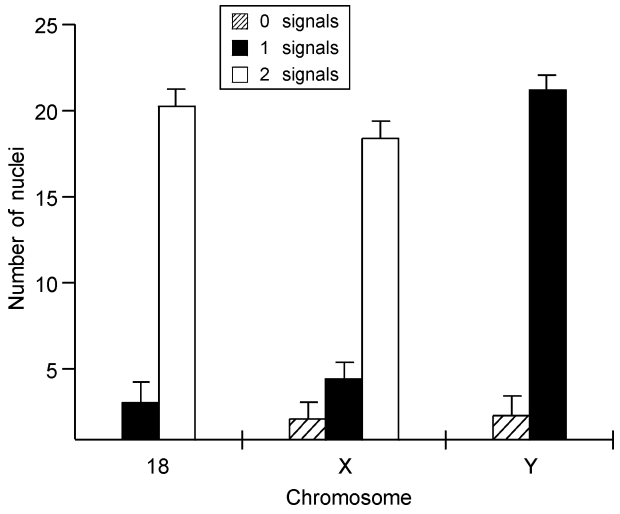

Fig. 2. Detection efficiency on uncultured amniotic fluid cells. Three separate hybridizations to the same specimen were evaluated 10-200 nuclei counted, respectively. For each probe, the average value is given.

panied by birth defects (1).

Cytogenetic analysis is now routinely offered only to women at increased risk of having a child with a chromosomal abnormality, the most common indications being advanced maternal age, high-risk levels of maternal serum alpha-fetoprotein (MSAFP), or combined levels of MSAFP, βHCG, and estriol, or family history. FISH makes it possible to identify specific chromosomes in interphase nuclei. Attempts at using FISH on uncultured amniotic cells are hampered by the nature of amniocytes, which are resistant to lysis; consequently only small proportion of amniotic nuclei are suitable for hybridization to chromosome probes.

The 13/21 DNA probe does not allow reliable scoring of chromosome 13 and 21 copy numbers in interphase nuclei. So, we have demonstrated that X, Y, and 18 centromeric DNA probes can be used for the detection of aneuploidies in interphase nuclei from uncultured amniocytes and compared the hybridization efficiency of the three probes on uncultured amniocytes. Our prospective study showed that the diagnosis of two aneuploidies such as 47,XXY, 45,X could be made available by FISH and confirmed by full karyotyping.

The detection of aneuploidies by FISH in interphase uncultured amniocytes has been described in several studies (2, 3, 8-10). The overall informative rate for FISH specimen in this study was 84%. A larger study showed that the overall accuracy and aneuploidy detection rate were 90% to 99.8%, and 73%, respectively in informative cases when FISH was compared with conventional karyotyping (5). The overall uninformative rate for FISH specimen in this study was 16%, compared with 10%

Table 3. Numbers of female, male, and sex aneuploides diagnosed by FISH and full karyotype among the 50 samples analysed

Sex chromosome constitution	FISH	Full karyotype
XX	19	23
XY	22	25
XXY	1	1
XO	1	1
Non-analyzable	7	0
Total	50	50

in the largest series of FISH specimens published to date (5). The uninformative FISH results in this study were the results of blood stained amniotic fluid, insufficient cells, and technical failure.

A little information exists at this time to guide obstetricians on the role of FISH on uncultured amniocytes in patients at high risk for aneuploidy.

The American College of Medical Genetics still recommends the clinical role of FISH on uncultured amniocytes to be considered investigational, and irreversible actions should not be taken on the basis of FISH alone. Therapeutic decisions may be appropriate. FISH on uncultured amniocytes confirms the interpretation of other diagnostic modalities such as ultrasonography and full metaphase karyotype. This would represent a more liberal interpretation of the current guidelines regarding the role of FISH on uncultured amniocytes in prenatal diagnosis (7).

The results of the present study support that FISH in uncultured amniocytes plays an important role in prenatal diagnosis for population at high risk for aneuploidy. However, our experience demonstrates some of limitations of current FISH protocols and the continued necessity for conventional karyotyping.

Further studies and specific and sensitive probes will be needed which will be more effective in noninvasive prenatal diagnosis.

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