

Amniotic Fluid Interphase Fluorescence in situ Hybridization (FISH) for Detection of Aneuploidy; Experiences in 130 Prenatal Cases

The major aneuploidies diagnosed prenatally involve the autosomes 13, 18, 21, and sex chromosomes X and Y. Fluorescence in situ hybridization (FISH) allows rapid analysis of chromosome copy number in interphase cells. We retrospectively reviewed 130 amniotic fluid interphase FISH analyses from January 1997 to December 2001. The review was done in order to assess the role of interphase FISH among the patients who were at the risk of fetal aneuploidies. The sample was considered to be aneuploid when 70% or more than the total number of hybridized nuclei displayed the same abnormal hybridization pattern for a specific probe. All of 130 cases but one met the criteria. The results were considered as informative and they were obtained in 24-48 hr. The overall detection rate for aneuploidies was 100% (2 cases of trisomy 21, 2 cases of trisomy 18, and 1 case of Turner syndrome). In comparison to cytogenetics, the rates of both sensitivity and specificity were 100%. The experiment demonstrates that FISH can provide a rapid and accurate clinical method for prenatal identification of chromosome aneuploidies. The experiment can also serve as an adjunctive test to help cytogenetics to reduce significant amount of emotional stress of patients and physicians through early decision making process.

Key Words : *In situ Hybridization; Fluorescence; Prenatal Diagnosis; Aneuploidy*

Ha-Jung Lim*, Yon-Ju Kim*,
Jae-Hyuk Yang*, Eun-Jeong Kim*,
June-Seek Choi*, Sang-Hee Jung*,
Hyun-Kyong Ahn*, Jung-Yul Han*,
Moon-Young Kim*, Kyu-Hong Choi*,
Jin-Mee Kim[†], Young-Mi Kim[†],
So-Yeon Park[†], Hyun-Mee Ryu*[†]

Department of Obstetrics and Gynecology*,
Laboratory of Medical Genetics[†], Samsung Cheil
Hospital and Women's Healthcare Center,
Sungkyunkwan University, School of Medicine,
Seoul, Korea

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Address for correspondence

Hyun-Mee Ryu, M.D.
Department of Obstetrics and Gynecology, Samsung
Cheil Hospital, 1-19 Mookjeung-dong, Choong-gu,
Seoul 100-380, Korea
Tel : +82.2-2000-7000, Fax : +82.2-2000-7477
E-mail : ryu97@samsung.co.kr

INTRODUCTION

Cytogenetic investigation is currently a standard prenatal diagnostic test. Prenatal cytogenetic analysis requires the isolation of metaphase chromosomes and takes 7-14 days for the final results (1). The length of time until the final results is considered to be unfavorable to patients and physicians because it can impose additional burden on them. Aneuploidies of 5 chromosomes (13, 18, 21, X, Y) account for 95% of the chromosomal aberrations that cause infants born with defects (2, 3).

Fluorescence in situ Hybridization (FISH) was first introduced as a potentially powerful tool in clinical cytogenetics (4, 5). During the late 1980s and the early 1990s, technical issues were the focus of research. Specific probes to determine which cell types are suitable for the use with FISH, and for more effective techniques for cell preparation and signal detection were studied intensively (6). Since 1993, the American College of Medical Genetics (ACMG) has taken the position that prenatal interphase FISH is worth investigating. In 1997, the FDA cleared the AneuVysion assay (Vysis, Inc.) to enumerate chromosome 13, 18, 21, X, Y for prenatal diagnosis (7). The use of interphase FISH for rapid prenatal diagnosis

of numerical chromosome abnormalities from direct preparations of amniocytes is now widespread (8, 9). Several major clinical studies have addressed the accuracy of prenatal detection of the most common aneuploidy by FISH (10).

FISH takes only 24-48 hr, costs about half of the conventional cytogenetic technique, and offers an opportunity to reduce anxiety through early decision making process. The purpose of the current study was to determine the accuracy of FISH in detection of aneuploidies in real clinical practice.

MATERIALS AND METHODS

The results of 130 amniotic fluid interphase FISH analyses performed from 1997 to 2001 at Laboratory of Medical Genetics at the Samsung Cheil Hospital were reviewed retrospectively. FISH was offered as an adjunct to the standard chromosome analysis at the request of the obstetrics staff and patients among 6,278 prenatal amniocenteses. Patients with prenatal procedure were interviewed for the consideration of rapid analysis by FISH as the replacement of the standard karyotyping. The limitations of the prenatal FISH analysis were also explained to the patients in detail.

For each specimen, 2-4 mL of clear amniotic fluid was applied. The FISH analyses were performed on uncultured amniocytes, using DNA probes specific for chromosome 13, 18, 21, X, Y. Alpha satellite probes were applied for chromosomes 13, 18, X, Y. While locus specific probes were implemented for chromosome 21 (Vysis, Illinois, U.S.A.). A minimum of 100 interphase nuclei with defined hybridization signals were enumerated for each chromosome by two different technicians.

Our analytic criteria were defined as disomic, when normal signal patterns were observed in $\geq 80\%$ of nuclei, and aneuploidy if $\geq 70\%$ of the nuclei had abnormal signal patterns. Results were reported as uninformative as the above criteria were not met. The standard cytogenetic analysis was based on the examination of G-banded chromosomes from at least 30 cultured metaphase cells from a minimum of two independent cultures. The results of interphase FISH analyses were compared to standard karyotype analyses from cultured cells.

The gestational age at amniocenteses and the indications for testing were recorded. The indications were classified as positive maternal serum screening test, abnormal ultrasonographic finding, advanced maternal age, and others.

FISH results were reported to the referring physician as they became available. The written report included: 1) a short description of the technique including the specific probes used, 2) the results of the test; numbers of signals for each tested chromosome, 3) the ploidy status of chromosome 13, 18, 21, X, Y, 4) the interpretation of the results, 5) the limitations of prenatal FISH analysis; FISH cannot detect structural chromosomal aberrations, mosaicism, and numerical abnormalities other than on chromosome 13, 18, 21, X, Y, 6) the recommendation to wait for the full cytogenetic results before implementing therapeutic action.

RESULTS

A total number of 130 prenatal samples (2%) were analyzed among 6,278 amniocenteses in our cytogenetic laboratory from January 1997 to December 2001. Maternal age varied from 25 to 39 (mean value was 34 yr). Gestational age at the

Table 1. Indication for prenatal interphase FISH studies

Indication	No.*	(%)	Aneuploidy [†]
Maternal serum screening positive	65	50	0
Abnormal ultrasound finding	49	37.9	5
Advanced maternal age	10	7.6	0
Rapid sexing (X-linked recessive disease)	2	1.5	0
Previous Down baby	4	3.0	0
Total	130	100	5

*Number of cases of FISH analyses performed. [†]Number of aneuploidy in interphase FISH studies.

time of the procedure varied from 14 to 23 (mean value was 17.5 weeks).

The indications for interphase FISH analyses are shown in Table 1. The main indications were positive maternal serum screening test (50%) and ultrasonographic abnormalities (37.9%). The indication for five aneuploidy were all fetal ultrasound abnormality.

The average length of time to obtain results varied from 24 to 48 hr for FISH, while the time varied from 2 to 3 weeks for conventional karyotyping. FISH results were reported to be significantly faster than those of karyotyping.

The results of FISH analysis in comparison with conventional cytogenetic karyotyping is stated in Table 2, 3. Among the indications of abnormal ultrasonographic finding, increased nuchal fold thickness (6 cases), heart anomaly (3 cases), omphalocele (1 case), and choroids plexus cyst (8 cases) showed normal results in FISH analyses. All of the 130 relevant normal and abnormal FISH results were confirmed by subsequent cytogenetic analysis with no false positives or negatives. There were 5 true positive (2 cases of trisomy 21, 2 cases of trisomy 18, and 1 cases of Turner syndrome) and 1 inconclusive FISH result (due to insufficient number of nuclei).

We demonstrated that uncultured amniotic cell derived from a Down syndrome fetus showed two signals for chromosome 13 and three for chromosome 21 (Fig. 1).

Compared to cytogenetic studies, the sensitivity of the test to detect trisomy 21, 18, 13 and sex chromosome aneuploidies was 100%. Chromosomal abnormality, such as 45, XY,

Table 2. Abnormal ultrasound findings and interphase FISH analyses

Abnormal ultrasound finding	No.*	FISH results	No. [†]
Increased nuchal translucency	12	Trisomy 21	1
Nuchal fold thickness	6	Normal	0
Cystic hygroma	6	45,X0	1
Heart anomaly	3	Normal	0
Omphalocele	1	Normal	0
Choroid plexus cyst	8	Normal	0
Multiple fetal anomaly	13	Trisomy 18	2
		Trisomy 21	1
Total	49		5

*Number of cases with abnormal ultrasound finding. [†]Number of chromosomal abnormality in FISH analysis.

Table 3. Chromosomal abnormality in cytogenetic study

Cytogenetic report	No.*
Trisomy 21	2
Trisomy 18	2
45,X	1
45,XY,t(13;14)pat	1
Total	6

*Number of chromosomal abnormality.

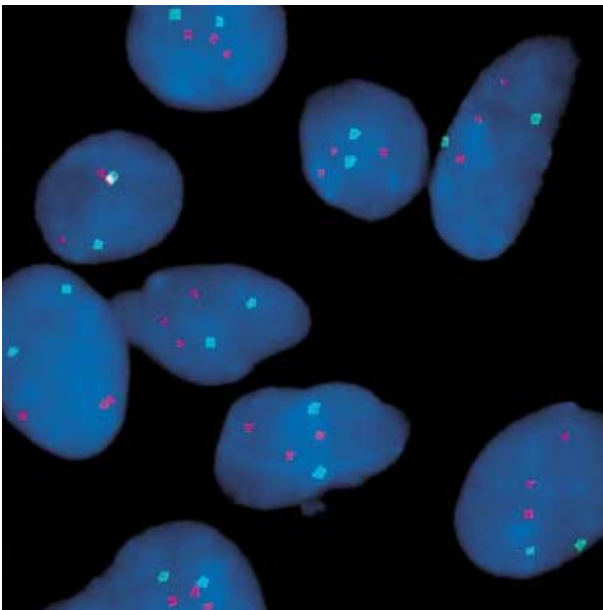


Fig. 1. Interphase nucleus from uncultured amniocytes by FISH shows two green (13 chromosome) and three red (21 chromosome) signals.

$t(13;14)pat.$ were not be detected by interphase FISH analysis.

DISCUSSION

Standard cytogenetic analysis of prenatal specimens detects chromosome aneuploidies and rearrangements with over 99.5% accuracy. However, standard karyotyping must be performed on metaphase cells, and therefore it takes culture time for several days. Especially, waiting for chromosome analysis can be place significant emotional stress for the patients and physicians (11). FISH performed on uncultured amniotic fluid cells, chorionic villous cells, and fetal blood cells with DNA probes specific for chromosomes 13, 18, 21, X, Y is used in several laboratories for rapid prenatal detection of aneuploidies, as an adjunct to routine metaphase cytogenetics (12-19).

The main indication of cytogenetic analysis was the abnormal ultrasonographic findings. It is proven to be effective especially for those patients referred near the time limit when termination of pregnancy is a considerable option (20). In our study, the main indications of rapid FISH analysis were positive maternal serum screening test (50%), abnormal ultrasonographic findings, and advanced maternal age with maternal anxiety. In our institution, routine ultrasound for fetal anatomy screening was performed around 20 weeks of gestation. When they showed abnormal results, we preferred to adapt percutaneous umbilical cord blood for rapid karyotyping. The decision was optimal, because it consumed only

3-5 days (21). The termination limit with lethal fetal anomaly was 23 weeks and 6 days in our institution.

Ward et al. reported that 9.8% of the FISH results were uninformative. The cause of the failed hybridization or problematic results is due to insufficient number of nuclei for analysis in one or more chromosomes (22). We had only one inconclusive result with insufficient number of nuclei in two chromosomes. This case was performed during the early stage of our study. The overall detection rate was almost 100%. The accuracy and reproducibility of FISH analyses was critically dependent upon the specificity and sensitivity of the probes (17, 18). The detection rate for aneuploidies was 100% in this study. Neither false-positive nor false-negative FISH results was found.

Feldman et al. reported that the incidence of chromosomal abnormalities in very high risk pregnancies is relatively higher (14%) than it is in low risk pregnancies. The most common finding of the abnormal chromosomes are 13, 18, 21, X, Y (76%) (20). The fetal anomaly detected by ultrasound is the most significant risk factor for chromosomal abnormalities. The incidence of aneuploidy of 13, 18, 21, X, Y was only 3.8% in our study. The incidence of chromosome abnormality, other than 13, 18, 21, X, Y, which would not have been detectable by FISH showed 14 cases (12.6%) in 111 abnormal results in Their group (23). In our group, there was only one case (14.3%) with clinically insignificant familial balanced translocation in 6 abnormal karyotype results.

The major problems found among most studies were the results of unsatisfactory criteria in interpretation of results. The different cut-off points for the proportion of cells with identical pattern of signals needed for diagnosis by FISH were between 50-70% in different studies. In this study, the cut-off points were 80% in disomic and 70% in aneuploidy. The total number of cells needed for diagnosis were relatively higher (100 cells) than that of other studies (50 to 100 cells). Two highly experienced technicians performed the microscopic evaluation.

Effective communication between the referring physician and cytogenetics laboratory is essential to improving the interpretation of FISH studies. An indication for a FISH test, accompanied by description of the specific fetal abnormalities diagnosed by ultrasound, may alert the clinical cytogeneticist, when a disomic, hybridization pattern is observed in cases likely to have autosomal or sex chromosomal aneuploidy. As shown in our study, rapid FISH was performed as an adjunct to routine metaphase cytogenetics on 130 prenatal samples submitted during the 5-yr period, according to the request of patients and physicians. The majority of aneuploidies associated with positive maternal serum screening test and abnormal ultrasound findings are standard trisomies from 13, 18, 21, X, Y, all of which would be diagnosed correctly and rapidly by FISH. This present study shows that FISH results can play an important role in counseling and decision making, especially for those patients

referred with severe emotional burden.

This experience demonstrates that FISH can provide a rapid and accurate clinical method for prenatal identification of chromosome aneuploidies as an adjunctive test to cytogenetics.

REFERENCES

- Ried T, Landes G, Dackowski W, Klinger K, Ward DC. Multicolor fluorescence in situ hybridization for the simultaneous detection of probe sets for chromosomes 13, 18, 21, X, and Y in uncultured amniotic fluid cells. *Hum Mol Genet* 1992; 1: 307-13.
- Robinson A, Bender BG, Linden MG, Salbenblatt JA. Sex chromosome aneuploidy: the Denver Prospective Study. *Birth Defects* 1990; 26: 59-115.
- Whiteman DAH, Kilnger K. Efficiency of rapid in situ hybridization methods for prenatal diagnosis of chromosome abnormalities causing birth defects. *Am J Hum Genet* 1991; 49(suppl): A1279.
- Cremer T, Landegent J, Bruckner A, Scholl HP, Schardin M, Hanger HD, Devilee P, Pearson P, van der Ploeg M. Detection of chromosome aberrations in the human interphase nucleus by visualization of specific target DNAs with radioactive and non-radioactive in situ hybridization techniques: diagnosis of trisomy 18 with L1.84. *Hum Genet* 1986; 74: 346-52.
- Julien C, Bazin A, Guyot B, Forestier F, Daffos F. Rapid prenatal diagnosis of Down syndrome with in-situ hybridization of fluorescent DNA probes. *Lancet* 1986; 2: 863-4.
- Philip J, Bryndorf T, Christensen B. Prenatal aneuploidy detection in interphase cells by fluorescence in situ hybridization (FISH). *Prenat Diagn* 1994; 14: 1203-15.
- Tepperberg J, Pettegnati MJ, Rao PN, Lese CM, Rita D, Wyandt H, Gersen S, White B, Schoonmaker MM. Prenatal diagnosis using interphase fluorescence in situ hybridization (FISH): 2-year multi-center retrospective study and review of the literature. *Prenat Diagn* 2001; 21: 293-301.
- D'Alton ME, Malone FD, Chelmow D, Ward BE, Bianchi DW. Defining the role of fluorescence in situ hybridization on uncultured amniocytes for prenatal diagnosis of aneuploidies. *Am J Obstet Gynecol* 1997; 176: 769-74.
- Lewin P, Kleinfinger P, Bazin A, Mossafa H, Szpiro-Tapia S. Defining the efficiency of fluorescence in situ hybridization on uncultured amniocytes on a retrospective cohort of 27407 prenatal diagnoses. *Prenat Diagn* 2000; 20: 1-6.
- Ryu HM, Kim ES, Lim SA, Park SY, Choi SK. Rapid Prenatal Diagnosis of Chromosomal Aneuploidy Using FISH. *Korea society of Med Genet meeting* 1995; 1: 43.
- Evers-Kiebooms G, Swerts A, van den Berghe H. Psychological aspects of amniocentesis: anxiety feelings in three different risk groups. *Clin Genet* 1988; 33: 196-206.
- Klinger K, Landes G, Shook D, Harvey R, Lapez L, Locke P, Lerner T, Osathanondh R, Leverane B, Houseal T. Rapid detection of chromosome aneuploidies in uncultured amniocytes by using fluorescence in situ hybridization (FISH). *Am J Hum Genet* 1992; 51: 55-65.
- Christensen B, Bryndorf T, Philip J, Xiang Y, Hansen W. Prenatal diagnosis by in situ hybridization on uncultured amniocytes: reduced sensitivity and potential risk of misdiagnosis in blood-stained samples. *Prenat Diagn* 1993; 13: 581-7.
- Divane A, Carter NP, Spathas DH, Ferguson-Smith MA. Rapid prenatal diagnosis of aneuploidy from uncultured amniotic fluid cells using five color fluorescence in situ hybridization. *Prenat Diagn* 1994; 14: 1061-9.
- D'Alton ME, Malone FD, Chelmow D, Ward BE, Bianchi DW. Defining the role of fluorescence in situ hybridization on uncultured amniocytes for prenatal diagnosis of aneuploidies. *Am J Obstet Gynecol* 1995; 176: 769-76.
- Bryndorf T, Christensen B, Vad M, Parner J, Brocks V, Philip J. Prenatal detection of chromosome aneuploidies by fluorescence in situ hybridization: experience with 2000 uncultured amniotic fluid samples in a prospective preclinical trial. *Prenat Diagn* 1997; 17: 333-41.
- Eiben B, Trawicki W, Hammams W, Goebel R, Epplen JT. A prospective comparative study on fluorescence in situ hybridization (FISH) of uncultured amniocytes and standard karyotype analysis. *Prenat Diagn* 1998; 18: 901-6.
- Jalal SM, Law ME, Carlson RO, Dewald GW. Prenatal detection of aneuploidy by directly labeled multicolored probes and interphase fluorescence in situ hybridization. *Mayo Clin Proc* 1998; 73: 312-137.
- Morris A, Boyd E, Dhanjal S, Lowther GW, Aitken DA, Young J, Menzies AL, Imrie ST, Connor JM. Two years' prospective experience using fluorescence in situ hybridization on uncultured amniotic fluid cells for rapid prenatal diagnosis of common chromosomal aneuploidies. *Prenat Diagn* 1999; 19: 546-51.
- Feldman B, Ebrahim SA, Gyi K, Flore LA, Evans MI. Rapid confirmation of previously detected prenatal mosaicism by fluorescence in situ hybridization in interphase uncultured amniocytes. *Genet Test* 2000; 4: 61-3.
- Yoo SI, Lee YH, Kim MY, Kim ES, Ryu HM, Kang HS, Hwang WY. Extended sonographic examination protocol for screening of fetal congenital heart disease at the Samsung-Cheil hospital in Korea. *Adv Obstet Perinatol* 1998; 9: 9-18.
- Ward BE, Gersen SL, Carelli MP, McGuire NM, Dackowaski WR, Weinstein M, Sandlin C, Warren R, Klinger KW. Rapid prenatal diagnosis of chromosomal aneuploidies by fluorescence in situ hybridization. *Am J Hum Genet* 1993; 52: 865-65.
- Thein AT, Abdel-Fattah SA, Kyle PM, Soothill PW. An assessment of the use of interphase FISH with chromosome specific probes as an alternative to cytogenetics in prenatal diagnosis. *Prenat Diagn* 2000; 20: 275-80.