Rapid prenatal diagnosis of chromosome aneuploidies in 943 uncultured amniotic fluid samples by fluorescence *in situ* hybridization (FISH)

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Purpose : Fluorescence *in situ* hybridization (FISH) on uncultured amniotic fluid cells offers the opportunity for rapid screening of aneuploidies and has become an integral part of the current practice in many clinical cytogenetics laboratories. Here, we retrospectively analyzed the results of interphase FISH in 943 amniotic fluid samples and assessed the efficiency of FISH for rapid detection of aneuploidies.
Methods : Interphase FISH for chromosome 13, 18, and 21 was performed in 943 consecutive amniotic fluid samples for rapid diagnosis of aneuploidies referred from 2004 to 2006. Karyotypes from standard cytogenetic analysis were compared to the FISH results.
Results : A total of 45 chromosomal rearrangements (4.8%) were found after conventional cytogenetic analysis of the 943 amniotic fluid. After exclusion of known familiar chromosomal rearrangements and inversions (2.1%, 20/943), 2.7% (25/943) were found to have chromosomal abnormalities. Of this group, 0.7% (6/943) were chromosomal abnormalities not detectable by FISH and 2.0% (19/943) were numerical abnormalities detectable by FISH. All 14 cases of Down syndrome (Classic type, 13 cases; Robertsonian type, 1 case) and 5 cases of trisomy 18 were diagnosed and detected by FISH and there were no false-positive or -negative results (specificity and sensitivity=100%).
Conclusion : The present study demonstrates that FISH can provide a rapid and sensitive clinical method for prenatal identification of chromosome aneuploidies. However, careful genetic counseling is essential to explain the limitations of FISH, including the inability to detect all chromosomal abnormalities and the possibilities of uninformative or false-negative results in some cases.

Key Words : Prenatal diagnosis, Fluorescence *in situ* hybridization (FISH), Aneuploidy, Amniocentesis, Genetic counseling

Introduction

Rapid prenatal detection of numerical chromosome abnormalities by fluorescence *in situ* hybridization (FISH) on uncultured amniotic fluid samples was introduced in 1992[1] and has become an integral part of the current practice in many clinical cytogenetics laboratories. Aneuploidies involving chromosomes 13, 18, 21 and sex chromosomes, accounts for 60-80% of abnormal karyotypes detected during prenatal diagnosis[2, 3]. FISH on uncultured amniotic fluid cells allows diagnosis with probes specific for these five chromosomes within 4-48h. Rapid diagnosis of fetal

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chromosome anomalies may facilitate clinical decision-making, especially when a fetal abnormality is detected late in pregnancy. 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 AneuVision assay (Vysis, Downers Grove, IL, U.S.A.) to enumerate chromosome 13, 18, 21, X and Y for prenatal diagnosis[4]. Several reports have described the effectiveness of interphase FISH in prenatal diagnosis[4-9]. The use of interphase FISH for rapid prenatal diagnosis of numerical chromosome abnormalities from direct preparations of amniocytes is now widespread[10, 11]. In the present retrospective study, we collected data on the results of interphase FISH in 943 amniotic fluid samples for 3 years (2004-2006) and assessed the efficiency of FISH to detect trisomies 13, 18 and 21, compared with conventional cytogenetic analysis.

Materials and Methods

A total of 943 amniotic fluid samples from 2004 to 2006 were subjected to interphase FISH as well as karyotyping. Amniocenteses were performed in various medical sites, but all samples were analyzed in the Seoul Clinical Laboratories. Clinical data, including age, gestational weeks and indications, were obtained from records at the request of the cytogenetics laboratory. Informed consent for genetic test was obtained from all subjects. The data that have been collected provided a detailed account of the number of amniocentesis results for the following indications: 1) AMA, that is, if the mother is >35 years old at the expected date of confinement; 2) previous history of fetus/child with chromosomal abnormalities and congenital anomalies; 3) family history of chromosomal abnormalities and congenital anomalies; 4) carrier of X-linked recessive disorder; 5) abnormal screening markers (α-fetoprotein, human chorionic gonadotropin and/or unsaturated estriol) in maternal serum; 6) abnormal ultrasonographic (US) findings; 7) history of missed or recurrent abortions and/or unexplained death in utero; 8) patient anxiety and 9) twins. Abnormal ultrasonographic findings were categorized as follows: fetal and placental malformations (single or multiple), abnormal amniotic fluid volume (polyhydramnios or oligohydrmanios) and intrauterine growth restriction. The distribution of fetal malformations comprised heart anomalies, diaphragmatic hernia, duodenal atresia, abdominal wall defects, fetal effusion/hydrops, hydrocephalus, mild ventriculomegaly and thickened nuchal fold (>6 mm).

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 and 21. For chromosome 13 and 21, LSI (locus- specific identifier) probes (Vysis, Downers Grove, IL, U.S.A.) were used, for chromosome 18, CEP (chromosome enumeration probe) was used (Vysis, Downers Grove, IL, U.S.A.). A minimum of 100 interphase nuclei with defined hybridization signals were enumerated for each chromosome by two different technicians. Hybridizations with fewer than 30 scorable nuclei, or fewer than 70% of nuclei with either aneuploid or aneuploid hybridization pattern, were considered uninformative. The conventional cytogenetic analyses of amniotic fluid samples were performed using an *in situ* culture method. The amniocytes were cultured in three Petri dishes in 2 mL of BIOAMF (Biological, Inc., Kibbutz Beit Haemek, Israel) as the basal medium supplemented with BIOAMF supplement (Biological, Inc., Kibbutz Beit Haemek, Israel), 1% 200 mM L-glutamine (Gibco, New York, USA), 100 U/mL penicillin (Biological, Inc., Kibbutz Beit Haemek, Israel.) and 100 ug/mL streptomycin (Biological, Inc., Kibbutz Beit Haemek, Israel.) using the technique described earlier[12]. Cultures were harvested when colonies were sufficient (at least 15 colonies), 6-10 days after seeding. Chromosomes were prepared in the usual manner[12]. Routine diagnosis was performed using the GTG-banding technique[13]. In some cases, analysis was completed by the C-banding technique. Cytogenetic diagnoses were based on examination of GTG-banded chromosomes from at least 20 cultured metaphase cells from a minimum of two independent culture dishes. The results of interphase FISH analyses were compared to conventional

cytogenetic analysis from cultured cells.

Results

Of the 943 samples, 43.1% had a maternal age between 30 and 34 years, which was the most common age group, followed by age 25-29 (242, 25.6%), 35-39 (233, 24.7%), older than 40 (46, 4.9%) and 20-24 (16, 1.7%). The aneuploidies were most frequently detected in age 30-34 (9, 47.3%) (Table 1). In declining order of incidence, amniocentesis was done at 17 gestational weeks in 37.2% of cases, 16 in 22.8%, 18 in 19.5%, 19 in 8.2% and less than 15 in 5.8%. The aneuploidies were most frequently detected in gestational weeks 18 (6, 31.5%) (Table 2). The indications for interphase FISH analyses are shown in Table 3. The main indication was an abnormal maternal serum screening test (84.5%), followed by AMA (4.3%), abnormal US findings (4.0%) and abnormal maternal serum screening combined with AMA (3.0%). Among indications, 15 aneuploidies (trisomy 21, 11 cases; trisomy 18, 4 cases) were referred for abnormal maternal serum screening test, 3 (trisomy 21, 2 cases; trisomy 18, 1 case) for abnormal US findings and one (trisomy 21, 1 case) for abnormal maternal serum screening combined with AMA. Of the 797 cases with abnormal US findings, 15 resulted in chromosomal aneuploidies, which showed the highest positive predictive value (8.3%) among indications.

Table 4 gives an overview of the 45 chromosomal rearrangements (4.8%) found after conventional cytogenetic analysis in the 943 amniotic fluid samples, and when compared to interphase FISH results. After exclusion of known familiar chromosomal rearrangements and inversions (2.1%, 20/943), 2.7% (25/943) chromosomal abnormalities found. Of this group, 0.7% (6/943) was chromo-

Table 1. Age Distribution

Maternal age (years)	No. of patients (%)	No. of aneuploidies detected by FISH			
		Trisomy 21	Trisomy 18	Trisomy 13	Total (%)
20-24	16 (1.7)	0	0	0	0 (0)
25-29	242 (25.6)	3	0	0	3 (15.8)
30-34	406 (43.1)	8	1	0	9 (47.3)
35-39	233 (24.7)	2	4	0	6 (31.6)
≥40	46 (4.9)	1	0	0	1 (5.3)
Total	943 (100)	14	5	0	19 (100)

FISH, Fluorescence *in situ* hybridization; No., Number

Table 2. Gestational Age Distribution

Gestational age (weeks)	No. of patients (%)	No. of aneuploidies detected by FISH			
		Trisomy 21	Trisomy 18	Trisomy 13	Total (%)
≤15	55 (5.8)	2	0	0	2 (10.5)
16	215 (22.8)	3	2	0	5 (26.3)
17	351 (37.2)	1	0	0	1 (5.3)
18	184 (19.5)	3	3	0	6 (31.5)
19	77 (8.2)	2	0	0	2 (10.5)
20	19 (2.0)	0	0	0	0 (0)
21	8 (0.8)	1	0	0	1 (5.3)
22	9 (1.0)	1	0	0	1 (5.3)
23	4 (0.4)	0	0	0	0 (0)
≥24	21 (2.3)	1	0	0	1 (5.3)
Total	943 (100)	14	5	0	19 (100)

FISH, Fluorescence *in situ* hybridization; No., number

somal abnormalities not detectable by FISH (Table 4, II A) and 2.0% (19/943) were numerical chromosomal abnormalities detectable by FISH (Table 4, II.B.). We had no uninformative results and the detection rate for aneuploidies was 100% in this study. All 14 cases of Down syndrome (Classic type, 13 cases; Robertsonian type, 1 case) and 5 cases of trisomy 18 were diagnosed detected by FISH and there were no false-positive or -negative results (specificity and sensitivity=100%). We demonstrated that uncultured amniotic cells derived from a Down syndrome and Edward syndrome fetus showed three signals for chromosome 21 and 18 (Fig. 1A and 1B).

Table 3. Indications for Prenatal Interphase FISH Studies

Indications	Aneuploidies detected by FISH		
	No. of patients ppppatients (%)	N	%
Advanced maternal age (AMA) (≥35 years)	41 (4.3)	0	0
Previous chromosomal abnormalities	6 (0.6)	0	0
Family history of chromosomal abnormalities	4 (0.3)	0	0
Abnormal maternal serum screening	797 (84.5)	15*	1.9
Abnormal ultrasonographic (US) findings	36 (4.0)	3**	8.3
Previous neonatal death or stillbirth	11 (1.2)	0	0
Patient anxiety	2 (0.2)	0	0
Twin	10 (1.1)	0	0
Abnormal maternal serum screening+AMA	27 (3.0)	1†	3.7
Abnormal maternal serum screening+Abnormal US findings	6 (0.6)	0	0
AMA+Abnormal US findings	3 (0.2)	0	0
Total	943 (100)	19	2.0

*11 cases of trisomy 21 and 4 cases of trisomy 18; **2 cases of trisomy 21 and 1 case of trisomy 18; † Trisomy 21
No., number

Table 4. Summary of 45 Chromosomal Rearrangements (4.8%) Found after Conventional Cytogenetics in 943 Prenatal Amniotic Fluid Samples

I. Balanced chromosomal rearrangements (N=20) (2.1%)			
Karyotype			N
46,XX,inv(9)(p12q13)			19
46,XX,t(2;18)(p11.2;q12.2)mat			1
II. Unbalanced chromosomal rearrangements (N=25) (2.7%)			
A. Chromosomal abnormalities (N=6) not detectable by FISH (0.7%)			
Karyotype			N
46,XY,del(4)(p15.1)			1
46,XX,del(22)(q13.1)			1
47,XY,+2[10]/46,XY[31]			1
46,XY,dup(9)(q21.2q22.1)			1
46,XX,add(5)(q35)			1
47,XX,+mar			1
B. Chromosomal abnormalities (N=19) detectable by FISH (2.0%)			
Karyotype	Conventional	FISH detected	Percentage
Trisomy 21	13	13	1.4
46,XY,der(14;21)(q10;q10),+21	1	1	0.1
Trisomy 18	5	5	0.5

N, number

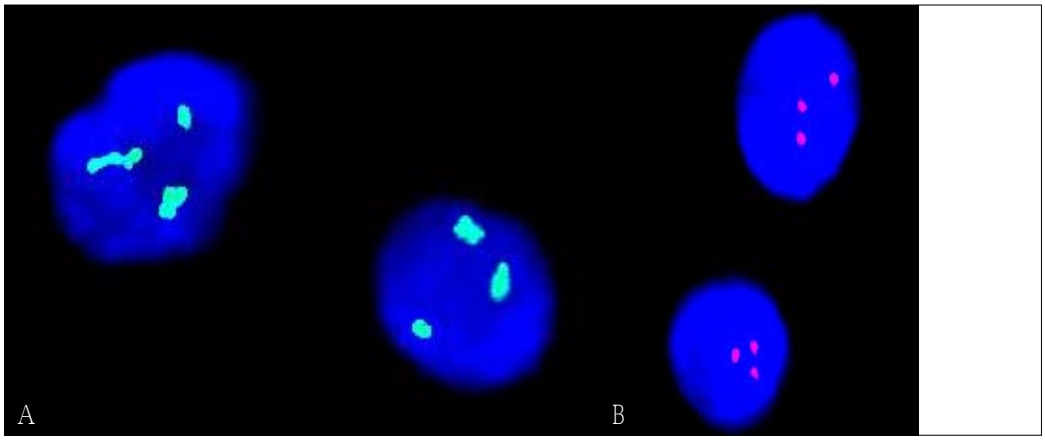

Fig. 1. Interphase nuclei from uncultured amniocytes by FISH shows (A) three green (18 chromosome) and (B) three red (21 chromosome) signals. Cytogenetic analyses show (A) 47,XX,+18 and (B) 47,XY,+21.

Discussion

Since the 1970s, karyotyping of fetal cells cultured from amniotic fluid has been the gold standard technique for the prenatal diagnosis of chromosomal disorders. However, a significant and well-known limitation of this technique is that cells have to be cultured, leading to a delayed result (commonly between 14 and 21 days). This waiting for chromosome analysis can be especially stressful for both the patients and the physicians[14]. Fluorescence in situ hybridization (FISH), performed in uncultured amniotic fluid cells with DNA probes specific for chromosomes 13, 18, 21, X and Y, has been used in several laboratories for rapid prenatal detection of aneuploidies, as an adjuvant to routine metaphase cytogenetics[1, 15-17]. We present the results of the application of interphase FISH for chromosome 13, 18, 21 in 943 consecutive amniotic fluid samples for the rapid prenatal diagnosis of aneuploidies performed during the period of 1 January 2004 to 31 December 2006.

In the 1980s, amniocentesis was used primarily for those in advanced maternal age groups, at least 35 years old. Other recent reports have shown that prenatal diagnosis of chromosomal disorders is still performed mainly for pregnancies at an advanced maternal age[18, 19]. Yang reported that the most common indication of amniocentesis for rapid prenatal diagnosis of chromosomal aneuploidies by FISH was due to advanced maternal age[20]. In the present study, the main indications of rapid FISH analysis were a positive maternal serum screening test (Table 3). In particular, this test has made remarkable progress as both a routine prenatal screening program and a detection technique in Korea. In studies by Yang et al[21], Tseng et al[22], and Karaoguz et al[23], abnormal US findings showed the highest detection rate for chromosomal abnormalities in prenatal diagnosis, at 6.5%, 8.9% and 5.3%, respectively. In the present study, of the 36 cases with abnormal US findings, 3 resulted in chromosomal aneuploidies, which showed the highest positive predictive value (8.3%) among the indications. Today, highly sensitive ultrasonic technology can detect many fetal anomalies, which eventually necessitate amniocentesis.

A total of 2.7% (25/943) chromosomal abnormalities were diagnosed after conventional cytogenetics; after exclusion of known familiar chromosomal rearrangements, i.e. balanced autosomal reciprocal or Robertsonian translocations and inversions, 2.1% (20/943) chromosomal abnormalities were identified. The present study found that FISH is a reliable technique for the rapid prenatal diagnosis of trisomy 21, as all 14 cases of Down syndrome were identified by interphase FISH and confirmed by conventional cytogenetics (sensitivity=100%), without false-positive results (specificity=100%). Five cases of Edward syndrome detected by FISH (Table 4B) were also confirmed by conventional cytogenetics and there were also no false positive

results. The reports on rapid detection of aneuploidy using FISH for prenatal diagnosis, consisting of various numbers of cases in Korea, have revealed that FISH can provide a rapid and accurate clinical method for prenatal identification of chromosome aneuploidies[20, 24-27]. Although FISH is reliable for detection of non-mosaic trisomies[11, 28], reports indicate that 15-30% of chromosome abnormalities detected by karyotyping would not be detected by FISH testing[2, 7, 29]. In present study, 0.7% (6/943) of the clinically significant chromosomal abnormalities (including unbalanced structural rearrangements, marker chromosomes and mosaic aneuploidies) were not detected by FISH (Table 4A). In addition to the chromosomal abnormalities described as being clinically important, there was a group of 20 cases with a familial, balanced translocation that would have been missed with rapid FISH alone. Although these are not significant in this pregnancy, they have the potential to result in unbalanced products in future pregnancies[30].

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[28]. The accuracy and reproducibility of FISH analyses was critically dependent upon the specificity and sensitivity of the probes[16, 17]. We had no uninformative results, and the detection rate for aneuploidies was 100%, in this study. The present results indicate good performance of the commercially available probe sets.

FISH analysis of uncultured amniocytes offers an informative result, in most cases, in 4-24 h. Rapid results may be crucial for important clinical decision-making in some cases and are helpful in decreasing the anxiety level in most patients with an abnormal maternal serum screening and increased risk for trisomy. However, it has been demonstrated that omitting karyotype analyses will lead to a significant number of false negative results related to other unbalanced abnormalities[3]. This is demonstrated by the residual risk (0.7%) of unbalanced abnormalities after a normal FISH result in present study. Clearly, there is no dispute that this FISH-based aneuploidy screening cannot detect aneuploidy for non-tested chromosomes, nor is it currently designed to detect euploid states with other cytogenetic abnormalities (e.g. translocation, inversion, marker chromosome).

The present study demonstrates that FISH can provide a rapid and sensitive clinical method for prenatal identification of chromosome aneuploidies as a complement to conventional cytogenetics. Careful genetic counseling is essential to explain the limitations of FISH, including the inability to detect all chromosomal abnormalities, as well as the possibility of uninformative or false-negative results in some cases.

한글요약

목 적 : 속 산전 염색체 이수성 진단을 위한 미배양 양수세포를 이용한 FISH 검사는 최근 많은 세포유전검사실의 중요한 업무 중의 하나가 되고 있다. 이에 본 저자들은 의뢰된 양수검체 943례에 대하여 산전 염색체 이수성 진단에 있어서 미배양 양수 FISH의 임상적 유용성을 알아보고자 한다.

방 법 : 2004년에서 2006년까지 의뢰된 943례의 양수검체에 대하여 염색체 13번, 18번, 21번에 대한 간기 FISH검사를 시행하였고, 산모의 나이, 임신주수와 적응증을 분석하였다. FISH 결과는 고전적 염색체 핵형분석과 비교분석하였다.

결 과 : 양수 검체 943례에 대해 염색체 핵형분석을 시행한 결과 45례(4.8%)에서 염색체 이상이 발견되었고 이를 간기 FISH결과와 비교하였다. 가족성 염색체 상호전좌와 역위 20례를 제외한 염색체 이상이 25례에서 발견되어 2.7%를 차지하였는데 그 중 6례(0.7%)는 FISH로 검출되지 않았고 19례(2.0%)는 FISH로 검출되었다. 핵형분석결과 다운증후군이 14례(Classic형 13례, 전좌형 1례)로 관찰되었다. 에드워드 증후군은 5례로 모두 FISH로 검출되었고 위양성, 위음성은 없었다(특이도와 민감도, 100%).

결 론 : 본 연구결과는 FISH검사가 염색체 이수성을 진단하는데 있어 고전적 핵형분석법을 보완할 수 있는 신속하고 예민한 방법이지만 FISH검사로 모든 염색체이상을 검출할 수 없으며 위음성결과를 보일수 있는 FISH 검사의 한계에 대하여 신중한 유전상담이 중요하다고 사료된다.

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