CYTOGENETIC EVALUATION OF CORDOCENTESIS MATERIALS IN THE PRENATAL DIAGNOSIS AND APPLICATION OF "FISH" AS AN ADDITIONAL TECHNIQUE

KORDOSENTEZ MATERYALİNİN PRENATAL TANI AMAÇLI SİTOGENETİK DEĞERLENDİRİLMESİ VE EK BİR TEKNİK OLARAK FISH UYGULAMASI

Ece KONAÇ, Ph.D., Meral YİRMİBEŞ KARAOĞUZ*, M.D,Ph.D., Mehmet Ali ERGÜN* M.D., Tuncay NAS** M.D., Sevda MENEVŞE, Ph.D.

Gazi University, Faculty of Medicine, Departments of Medical Biology and Genetics, Medical Genetics* and Obstetrics and Gynecology, Ankara, Turkey**
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ABSTRACT

Purpose: *To evaluate the cytogenetic results of cord blood samples and apply fluorescence in situ hybridization (FISH) as an additional technique to "46,XX cytogenetically diagnosed cases" in order to confirm the karyotypes or to verify the occurrence of a Y chromosome component.* ***Methods:*** *111 cord blood specimens were cultured for prenatal karyotyping. Of the 66 "46,XX kartoyped" materials, 20 of them were painted with X (DXZ1) and Y (DYZ3) chromosome ?-satellite probes by FISH.* ***Results:*** *Of the 111 cases, 106 (95.5%) were karyotypically normal, and 5 (4.5%) were abnormal (two regular and one mosaic trisomy 21, one X mosaicism and one pericentric inversion of chromosome 9). In one of the 20 "46,XX" karyotyped cord blood specimens, 104 of 500 cells (20.8%) revealed one X signal and one Y signal.* ***Conclusion:*** *The evaluation of abnormal and 46, XY karyotypes in cord blood samples gave accurate results but "46,XX" karyotypes must be confirmed with an additional technique like FISH for an accurate cytogenetic evaluation of specimens.*

Key Words: *Cordocentesis, Karyotypes, Fluorescence İn Situ Hybridization (FISH), Y Chromosome Component.*

ÖZET

Amaç: *Bu çalışmanın amacı kord kanı örneklerinin sitogenetik sonuçlarının değerlendirilmesi ve "sitogenetik olarak 46,XX" tesbit edilen olgularda floresan insitu hibridizasyon tekniği (FISH) uygulaması ile karyotipin doğrulanmasının sağlaması veya Y kromozomuna ait komponentin varlığının gösterilmesidir.* ***Metodlar:*** *Prenatal tanı amaçlı 111 kord kanı kültüre edildi. "Karyotipi 46,XX" olan 66 olgunun, 20'sine X (DXZ1) ve Y (DYZ3) kromozomlarına ait α -satellit problar ile FISH tekniği uygulandı.****Bulgular:*** *Toplam 111 olgunun 106'sında (%95.4) karyotip normal iken, 5 (%4.5) olguda anomali belirlendi (iki regüler, bir mozaik trizomi 21, bir X kromozom mozaisizmi bir de 9. kromozomda perisentrik inversiyon). Karyotipi "46,XX" olan 20 olgudan bir tanesinde, analiz edilen 500 hücrenin 194'ünde (%20.8) hem X kromozomuna ait, hem de Y kromozomuna ait sinyal izlenmiştir.* ***Sonuç:*** *Sonuç olarak kord kan örneklerinde anomalili veya 46,XY karyotipinin belirlenmesi sonucun güvenilir olduğunu düşündürmesine karşın, "46,XX" karyotiplerinde sitogenetik değerlendirmenin doğruluğunun teyidi için FISH gibi ek bir tekniğin uygulanması gerekmektedir.*

Anahtar Kelimeler: *Kordosentez, Karyotip, Floresan in Situ Hibridizasyon (FISH), Y Kromozom Komponenti.*

INTRODUCTION

The overall frequency of chromosomal abnormalities has been reported to be about 1 in 160 in live-born infants (1). This frequency of chromosomal abnormalities in the population led to the evolution and inevitable use of prenatal diagnostic methods (2).

Cordocentesis (umblical cord blood sampling) is a method commonly used in the second and third trimesters of pregnancy to diagnose several prenatal disorders (3). No problems are encountered in the standard cytogenetic diagnosis of karyotypes of normal male cord blood samples (46, XY) or of those of abnormal ones. However, the problem of misdiagnosis may occur in the karyotypes of normal female cord blood samples (46, XX). This

is mainly due to the fact that it is possible for the normal female or male cord blood to be contaminated by maternal cells (4,5). Various tests, such as the Apt test/hemoglobin alkaline denaturation test, are conducted to understand whether the fetal blood samples obtained by cordocentesis are pure or contaminated (6,7). Nevertheless, these tests are not fully sufficient for reliable assessments. Therefore, in addressing the issue of contaminating maternal cells (in male fetuses), an additional technique, like fluorescence in situ hybridization (FISH), can be performed to evaluate the occurrence of a "Y chromosome component" in "46,XX karyotyped" ones.

The present study was a preliminary prospective trial with FISH as an "additional" technique for "46,XX" cytogenetically diagnosed cord blood specimens besides the presentation of the distribution of abnormal karyotypes.

MATERIALS AND METHODS

Umbilical Cord Blood Culture: Cordocentesis was performed in 111 cases for prenatal karyotyping between June 2002 and June 2004. Cord blood samples (2 ml) were collected and a slightly modified version of the tissue culture procedure described by Verma and Babu was performed (8). Karyotypes were prepared proceeding from two independent cultures of the same sample. The cultures were harvested at 68-72 h, after a 75 min incubation period with 0.1 µg/ml of colchicine. The cell pellets were treated with hypotonic KCl solution (0.075 M) at 37 °C for 30 min, then fixed in a fresh solution of methanol/glacial acetic acid (3:1) and finally washed three times in fresh fixative. All karyotypes were examined with a slightly modified Giemsa banding technique (9). At least twenty metaphases were analyzed for each sample and in cases of mosaicism or abnormal results, thirty or more metaphases were evaluated.

Fluorescence In Situ Hybridization (FISH): During the FISH analysis, X (DXZ1) (spectrum green) and Y (DYZ3) (spectrum red) chromosome ?-satellite probes were used by employing the manufacturer's protocol (10). The numbers of X and/or Y signals were counted, using a Zeiss epifluorescence microscope with a triple filter set, in 500 intact, nonoverlapping interphase and/or metaphase cells. In cases of detection of Y signals in the 46, XX karyotyped samples, at least fifty metaphases were analyzed cytogenetically.

RESULTS

Obstetric histories and cytogenetic results of the 111 cordocentesis cases are given in Tables 1 and 2. Of the 111 cases, 106 (95.5%) were karyotypically normal (66 of them 46,XX; 40 of them 46,XY), and 5 (4.5%) were abnormal (Table 1). The mean maternal ages were 29.9 and 29.4 and the mean weeks of gestation were 21.9 and 24 in normal and abnormal karyotyped cases, respectively (Table 1). The prenatal indications for cordocentesis included 37 cases of fetal ultrasound abnormality, 34 cases of risk suggestive triple screening test, 34 cases of advanced maternal age, 4 cases of intrauterine growth retardation and 2 cases of previous congenital abnormality (Table 2). In three of 37 (8.1%) fetal ultrasound abnormal cases, cytogenetic analyses revealed regular trisomy 21 and pericentric inversion of chromosome 9 (Table 2). In two of 34 (5.9%) triple screening test positive cases, cytogenetic evaluations showed one mosaic trisomy 21 and one X mosaicism (Table 2).

Of the 66 normal 46,XX karyotyped cord blood specimens, FISH was performed on 20 samples in order to confirm the accuracy of cytogenetic evaluations (Table 1). In only one of the 20 specimens, 104 of the 500 cells (20.8%) revealed one X signal and one Y signal (Fig. 1a and 1b).

DISCUSSION

Cordocentesis has become a widely accepted procedure for invasive fetal diagnosis and in fetuses at high risk of chromosomal aberrations, for rapid karyotyping, and it has significant positive impacts on fetal outcomes (11-14). In the present study, 5 (4.5%) abnormal karyotypes were detected with this application, which was slightly lower than the rates reported previously (5.2-18%) (11,15).

Malformations are frequently associated with chromosomal abnormalities, and prenatal karyotyping, using cordocentesis, is dispensable for further perinatal care, when sonography reveals fetal anomalies (16,17). In the present

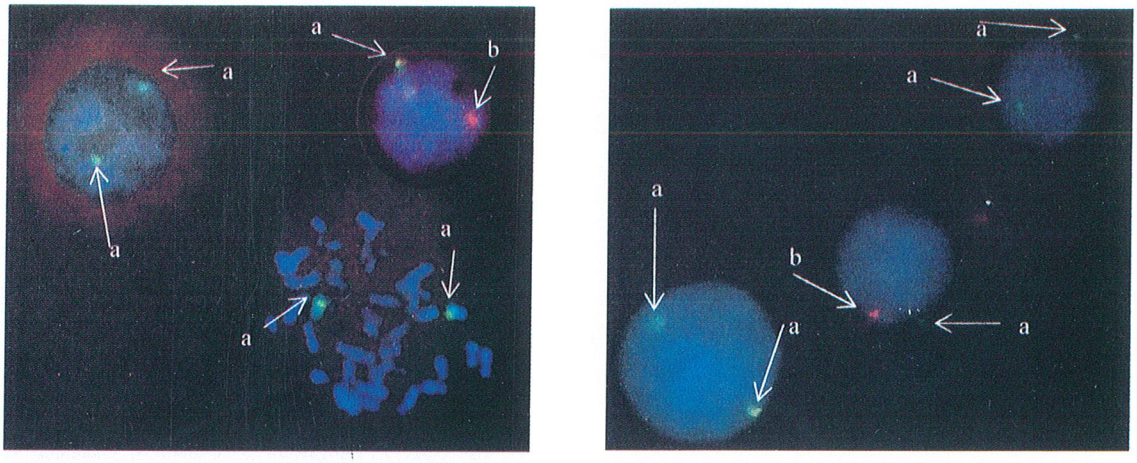

Fig. 1A;B: FISH analysis of the "46, XX karyotyped" cord blood sample indicating the presence of a Y chromosome component (at 1000X magnification). DXZ1 (spectrum green) and DYZ3 (spectrum red) a-satellite probes in the metaphase spread and interphase nuclei. A) X signals (a) and Y signal (b) in two interphase nuclei and one metaphase plaque. B) X signals (a) and Y signal (b) in three interphase nuclei.

study, the most frequent indication was malformation and chromosomal aberration was found in three out of 37 cases (8.1%) having malformations detected by fetal sonography, concordant with Murotsuki et al. (7%, in six out of 86 cases) (Table 2) (11). The second most frequent indication, risk suggestive "triple screening test", revealed two abnormal results (Table 2). In most studies, the mean gestation age was reported as 28-30 weeks, a departure from our average (the mean was 22.1 in our study) (18-21) (Table 1).

The "46,XX karyotypes" of the fetal blood samples cannot always indicate normal female fetuses as there is a risk of false-negative results due to the unverified maternal cell contamination (7,13,15). For this reason, an additional technique like FISH is required to confirm the accuracy of the cytogenetic diagnosis or to confirm inaccurate cytogenetic test results most likely attributable to maternal cell contamination of a male fetus (22). In the present study, in one of the 20 46,XX cytogenetically diagnosed cases, one X signal and one Y signal were detected, which probably pinpoint the maternal cell

Table-1: Obstetric histories and cytogenetic results of cordocentesis materials.

	Number	Maternal Age (year)		Weeks of gestation	
		Mean	Range	Mean	Range
Normal karyotypes	106				
	(46, XX*: 66 cases, (46,XY: 40 cases)	29.9	20-44	21.9	16.5-37
Abnormal karyotypes	5	29.4	23-38	24	20-28
Total	111	29.9	20-44	22.1	16.5-37

* FISH was performed on 20 materials of the 66, 46,XX karyotyped materials.

Table-2: Abnormal cytogenetic results and prenatal indications of the cordocentesis materials.

Indications for prenatal cytogenetic diagnosis of cordocentesis materials	Number of cases (%)	Abnormal cytogenetic results
Fetal ultrasound abnormality	3/37 (8.1%)	47, XX, +21 (2 cases) *46, XY, inv (9) (p11;q13) (1 case)
Risk suggestive "triple screening test"	2/34 (5.9%)	46, XX/ 47, XX, +21 (1 case) ** 45, X/ 47, XXX (1 case) ***
Advanced maternal age	34	-
Intrauterine growth retardation	4	-
Previous congenital abnormality	2	-

*Karyotypically abnormal, phenotypically normal case.

** The fetal karyotypes of the CS were 46,XX (75%) in 45 clones and 47,XX,+21 (25%) in 15 clones.

*** The fetal karyotypes of the CS were 47,XXX (78%) in 39 clones and 45,X (22%) in 11 clones.

contamination of a male fetus (advanced ultrasound analysis and the birth of a male fetus revealed the accuracy of FISH) (Fig. 1a and 1b).

Finally, cordocentesis is an efficient procedure for fetal assessment in "normal 46,XY" and "abnormal" karyotypes, but in "normal 46,XX cytogenetic diagnosed" cases an additional technique, like FISH, is required to confirm the cytogenetic results or to evaluate the occurrence of a Y chromosome component. In conclusion, this study illustrates the application of both routine and molecular cytogenetic procedures in establishing an accurate cytogenetic diagnosis.

Correspondence to: Meral YİRMİBEŞ KARAOĞUZ, M.D,Ph.D
Gazi Üniversitesi Tıp Fakültesi
Tıbbi Genetik
Anabilim Dalı
Beşevler
06510 ANKARA - TÜRKİYE
Phone: 312 - 202 46 44
Fax: 312 - 212 46 47
E-mail: karaoguz@gazi.edu.tr

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