Prenatal Diagnoses with Amniocentesis and Cordocentesis: Evaluation of 181 Cases

Melih Atahan Güven[1], Serdar Ceylaner[2]

[1]Department of Gynecology and Obstetrics, Faculty of Medicine, Kahramanmaraş Sütçüimam University, Kahramanmaraş
[2]Genetik Center, Zekai Tahir Burak Training and Research Hospital, AnkaraDepartment of Gynecology and Obstetrics, Faculty of Medicine, Fırat University, Elazığ

Abstract

Aim: The aim of this study was to evaluate the results of invasive prenatal diagnostic procedures, amniocentesis and cordocentesis, performed in our clinic during 2002 and 2004.

Methods: Prenatal invasive diagnostic procedures were performed during 2002 and 2004 period of time due to high risk in triple screening test (≥ 1/270), advanced maternal age (≥ 35), anomaly detection during obstetric ultrasonography, and other indications were evaluated. Retrospectively, 150 cases (16-21 weeks), 31 cases (19-21 weeks) that were evaluated by amniocentesis and cordocentesis, respectively.

Results: Tissue cultures were successful in 150 of 153 cases (%98) evaluated by amniocentesis and %100 successful in all of 31 cordocentesis. Chromosomal abnormality rate was 3.8% (7/181) in all cases with successful cultures. The largest group of indications was high risk in triple screening test (78/181) in which the percentage of chromosomal abnormalities was 3.8% (3/78). There was no chromosomal abnormality detected in 49 (%27) cases with the indication of only advanced maternal age. In the evaluation of 23 (%12) cases with ultrasound abnormalities, 3 cases (13%) were detected with chromosomal abnormalities. Pregnant women presented with other indications (%18) in whom only one of 31 cases (3.2%) was chromosomal anomaly.

Conclusion: The rate of producing a successful tissue culture was %98 in our first retrospective study on fetal karyotype. There was no fetal loss in respect to the invasive procedure. Prenatal diagnosis must be performed in all cases with ultrasound abnormalities. Cases with low risk in triple screening test should be evaluated

Keywords: Amniocentesis, cordocentesis, fetal karyotype, chromosomal abnormalities.

Amniyosentez ve kordosentez ile prenatal tanı: 181 olgunun değerlendirilmesi

Amaç: Bu çalışmanın amacı 2002 ve 2004 yılı boyunca kliniğimizde uygulanan amniyosentez ve kordosentez sonuçlarının değerlendirilmesidir.

Yöntem: 2002 ve 2004 yıllarında üçlü test yüksek risk (≥ 1/270), ileri maternal yaş (≥ 35), ultrasonografide anomali izlenmesi ve diğer sebeplerle uygulanan karyotip tayini amaçlı girişimsel işlemler değerlendirildi. Gebeliğin 16-21. haftaları arasında uygulanan 150 amniyosentez ve 19-28. gebelik haftaları arasında uygulanan 31 kordosentez olgusunun yer aldığı, toplam 181 hastanın verileri retrospektif olarak değerlendirildi.

Bulgular: Gerçekleştirilen 153 amniyosentez girişiminden, 150'sinde doku kültürü başarılı oldu. Amniyosentez için kültürde başarı oranımız %98 idi. 31 kordosentez girişimimizin tümünde kültürde üreme başarısı sağlandı. Girişimsel işlemin uygulandığı ve üreme sağlanan olgularımızda kromozom anomali oranı %3.8 idi (7/181). Karyotip tayini amaçlı yapılan girişimsel işlemlerde endikasyon olarak en büyük dilimi, üçlü testte yüksek risk çıkan grup oluşturdu. Üçlü testte yüksek risk tespit edilen 78 (%43) olguya, karyotip tayini amaçlı girişimsel işlem uygulandı ve 3 (%3.8) olguda kromozom anomalisi izlendi. Sadece ileri maternal yaş sebebiyle değerlendirilen 49 (%27) olguya karyotip tayini amaçlı girişimsel işlem uygulanmasına rağmen, hiçbir olguda kromozom anomali-

Corespondence: Dr. Selahattin Kumru
Fırat Üniversitesi Tıp Fakültesi, Kadın Hastalıkları ve Doğum Anabilim Dalı, Elazığ

si izlenmedi. Ultrasonografide anomali/ anomaliler izlenen 23 (%12) olgunun değerlendirilmesinde, 3 (%13) kromozom anomalisi tespit edildi. Girişimsel işlemin uygulandığı diğer 31 (%18) olgudan, sadece birinde (%3.2) kromozom anomalisi izlendi.

Sonuç: Geriye dönük olarak incelediğimiz ilk karyotipleme serimizde kültür üretme başarı oranımız %98 idi. Girişimsel işlemlere bağlı fetal kayıp izlenmedi. Ultrasonografide anomali izlenen tüm olgulara prenatal tanı uygulanması ve üçlü test sonucunda düşük risk saptanan hastalara, ilerleyen haftalarda detaylı ultrasonografi yapılması kromozom anomalilerinin yakalanmasına yardımcı olmaktadır.

Anahtar kelimeler: Amniyosentez, kordosentez, fetal karyotip, kromozom anomalisi.

Introduction

It is possible to get information about fetal karyotype by means of interferential processes used in prenatal diagnosis. Amniocentesis was first used in 1950 for sex determination and it was first included to clinical practice by making karyotype determination from fetal cells.[1] In the last 30 years, most frequent indication for amniocentesis has been advanced age pregnancy. Except this indication, amniocentesis is being used for diagnosis of illnesses related with DNA analysis (like hemoglobinopathies), enzymatic analysis determination (diagnosis of metabolic illnesses) and for determination of congenital infections by PCR (Polymerase Chain Reaction). Multi-centered studies done up to now showed the reliability of second trimester amniocentesis for mother and fetus.[2-4] Widespread usage of scanning tests in the last decade and becoming widespread of determination by ultrasonography for diagnosis of chromosome anomalies increased demand for amniocentesis.

Cordocentesis was first included to clinical practice by using for fetal blood, asphyxia, karyotype and infection determination in the end of 80s following the application with ultrasonography in 1984.[5,6] Though it is known that fetal mortality related with interferential procedure at pregnancies having problem may be higher; general average is accepted as 1-2%.[7,8]

In this work, we evaluated the results of interferential processes done for karyotype determination in between 2002 and 2004.

Methods

138 pregnants which had karyotype determination by amniocentesis and cordocentesis for the purpose of prenatal diagnosis in Obstetrics Clinic of Medical Faculty of Kahramanmaras Sutcu Imam University were determined retrospectively in terms of interference indications, fetal prognosis, cell culture success and genetic results. All cases and their spouses were informed orally about procedure technique and their possible complications before interference. Written consent forms were taken from couples before the application who accepted the interference. All patients were checked in terms of general blood biochemistry, hepatitis porter, Rh incompatibility. All interferential processes were performed by one operator (M.A.G). ALOKA 4000 Prosound Model (Aloka Co., Ltd., Tokyo) 3.5 MHz transabdominal probe was used at interferences. After a detailed systematic ultrasonography determination and placenta localization; 1 ml amnion fluid was taken for each week from an area far from placenta by 20-21 G injector in between 16^{th} – 21^{st} gestational week by obeying classical amniocentesis rules.[9,10] Fetal loss occurred within two weeks following the interference was determined as a complication belonging to the process. Cordocentesis was done by taking 2 cc blood by 20-21 G needle to injector which 0.5 cc heparin existed from a free cordon concerning with localization of placenta or from 1-2 cm far from the point that cordon enters to placenta in between 19th-28th gestational week.[11]

Triple test determination was done by measuring AFP, HCG, Ostriol levels in maternal blood in between 15^{th}-20^{th} gestational week. Biparietal diameter measurement was done in order to determine gestational week of the fetus. Interferential process with the purpose of karyotype determination related with gestational week and/or placental localization was applied to pregnants who had ≥1/270 risk

of bearing baby with Down syndrome.

The material was sent to Intergen-Ankara genetic laboratory in order to cytogenetic determination of amnion fluid and fetal blood. While amniotic fluids were worked by flask technique, cordocentesis were cultured by classical methods in RPMI medium. At least 20 metaphase plates were examined by using image analysis system in order to determine numeric and structural disorders in chromosome at all patients.

Results

Average gestational week of cases which had interferential process and indications of amniocentesis and cordocentesis cases are shown in Table 1. No result was obtained from three of 153 amniocentesis cases due to previous bleeding and infection related with it, thus 150 cases were taken into consideration. Karyotype resulting rate was found as 98% (150/153). Cell cultures succeeded in all cases (n:31) being applied cordocentesis. No complication (fetal loss) related to interference was found in cases which had interferential process. Chromosome anomaly rate was 3.8% (7/181) in our entire series. Four of cases (4/13, 12.9%) which were observed chromosome anomaly were found by cordocentesis and three of them (3/150, %2) were found by amniocentesis.

High risk at triple test was the most frequently seen indication within amniocentesis cases. Interferential process for the purpose of karyotype determination was applied to totally 78 (43%) cases after test and chromosome anomaly was observed in 3 cases (3.8%). There was age risk in addition to high risk at triple test in 10 of 78 cases. No chromosome anomaly was observed in these 10 cases.

Interferential process was only applied to 49(27%) cases which were evaluated due to advanced maternal age (35-46). No chromosome disorder was seen in any case. In the determination of 23 cases (12%) which were found anomaly/anomalies in ultrasonography, 3 (13%) chromosome anomalies were found.

Chromosome anomaly was observed only in 1 case (3.2%) within 31 cases which were applied interferential process for other reasons. Short femur, humerus and increased nuchal edema (6.1 mm) was found at 22nd gestational week of the case (trisomy 21) which was observed chromosome anomaly though high risk was not found (1/540) at triple test done in 17th gestational week.

Distribution of patients applied interferential process for other reason is shown in Table 2.

Cases found chromosome anomaly were ended by demand of families. Qualities of these cases are shown in Table 3.

Discussion

Amniocentesis is the oldest well-known prenatal diagnosis method which is used most in the practice. Amniocentesis for the purpose of karyotype determination is frequently applied within 16th-20th gestational week. It was shown that amniocentesis applied in this period and rate of fetal loss related with interference brought 1% additional risk as to group which was not applied amniocentesis.4 No fetal loss related with interference in our work in which totally 150 amniocentesis cases and 31 cordocentesis cases were evaluated by only one operator.

Table 1. Indications, average gestational weeks and ages of cases applied amniocentesis and cordocentesis.

	Indication	High risked triple test	Advanced maternal age	Anomaly/anomalies found in ultrasonography	Other reasons
N - %	69 (%88)	48 (%98)	11 (%48)	22 (%71)	
Amniocentesis	Average gestational week	17.7±1.8	16.7±0.5	18.3±2.4	19.7±3.1
	Average age	30.9±3.2	37.6±1.8	23.7±2.8	29.3±1.9
N - %	9 (%12)	1 (%2)	12 (%52)	9 (%29)	
Cordocentesis	Average gestational week	20.7±1.1	23	24.5±1.9	24.1±2.8
	Average age	25.7±2.2	36	25.7±3.4	25.4±2.1

Table 2. Distribution of cases applied interferential process for other reasons.

Indication	Existence of chromosome anomaly determinant in US more than one	Being more than 1≥270 of riskof Tr 18	Isolated VM	Tx Ig M height + not resulting of avidity test	Baby case with Tr 21 syndrome	Being more than 1≥270 of risk of double test	Serious IUGR	Increased nuchal second trimester	Total
Cases applied CS	9	3	1	4	2	3	0	0	22
Cases applied CC	2	0	4	0	0	0	2	1	9
Total	**11**	**3**	**5**	**4**	**2**	**3**	**2**	**1**	**31**

AC: Amniocentesis, **CC:** Cordocentesis, **US:** Ultrasonography, **VM:** Ventriculomegaly , **Tx:** toxoplasmosis, **Tr:** Trisomy, **Double test:** Combination of maternal age and nuchal thickness with PAPPA + HCG, **IUGR:** Intrauterine growth restriction

When all amniocentesis cases were evaluated, only 3 of 153 cases had culture failure. Culture success from fetal cells we obtained was 98%. Our success rate was similar to the result of 99% of Cengizoglu et al.[12] Contamination of samples which was showed as the reason of low culture success in the studies of Yayla et al[13] was also the most important reason for cases having no reproduction within our series.

Chromosome anomaly rate of 3.2% (n:7) obtained from 181 cases which were performed by all interferential processes was compatible with the results that Yayla et al, Basaran et al1[2,14] obtained. Four of chromosome anomalies observed in our series were obtained by cordocentesis (4/31, 12.9%) and three of them were obtained by amniocentesis (3/150, 2%). This chromosome failure rate we obtained in interferential processes was partially similar with rates of Yazicioglu et al[15] which were 5.8% in amniocentesis group and 15.25% in cordocentesis group in their work performed in a near past.

Diagnosis of fetal anomalies can be performed by a detailed ultrasonographic examination in second trimester. 3 of 23 cases we found fetal anomaly by ultrasonography were observed chromosome anomaly (13%). This rate was within the rates 8.7%-27.1% mentioned in literature.[13,16]

Maternal serum biochemical scanning test (triple test) is kind of test which is done by basing on some biochemical determinants which are in maternal serum in between 15th-20th gestational weeks and secreted by mother-fetus unit and it is sensitive about 60% with 5% mistake rate for Down syndrome.[17] When interferential process with purpose of prenatal diagnosis are applied to pregnants older than thirty-five, 25-40% of cases with Down syndrome can be diagnosed.[18] Singh et al stated the sensitivity of triple scanning test done in second trimester of advanced maternal age cases for Down syndrome as 92.3% with 0.8% mistake rate.[19]

No chromosome anomaly was found in 49 cases within our series which were applied interferential process due to only advanced maternal age. Additionally, karyotype of 10 cases was normal which had high risk for Down syndrome in triple test and had age risk. Taner et al found Down syndrome risk as 1.11% and chromosome anomaly risk as 5.84% in their study in which they evaluated amniocentesis results in 359 advanced age gestation.[20] In our work, the reason for not finding chromosome abnormalities in cases including advanced maternal age + risked triple test which were in only advanced maternal age group can be explained by the minority of our case count.

Triple test risk was 1/540 of the case in 17th gestational week which was observed femoris, humerus and increased nuchal edema (6.1 mm) at 22nd gestational week within cases that we applied interferential process for other reasons even the cordocentesis result was found as Down syndrome, thus genetic ultrasonography to be done in the next periods of gestation is important.

Table 3. Qualities of cases which were applied amniocentesis and cordocentesis and which were found chromosome anomaly.

Karyotype	Age	Indication	Interferential process and its week	Ultrasonographic qualities	Prognosis
47, XX, +21 (Down syndrome in regular type)	35	Triple test risk 1/564, determinants increasing the possibility of chromosome anomaly in ultrasonography	Cordocentesis - 22	Short femoris and humerus, increased nuchal edema	Terminated
47, XX, +21 (Down syndrome in regular type)	30	Determination of 1/20 risk in triple test	Amniocentesis - 18	Echogenic intestine in light type	Terminated
47, XX, +21 (Down syndrome in regular type)	28	Determination of 1/140 risk in triple test	Amniocentesis - 17	Not available	Terminated
46, XX, der (15) add (8qter_8q21.2:15 pter_15qter)mat (Partial trisomy 8q)	23	Determination of 1/160 risk in triple test	Cordocentesis - 23	Not available	Terminated
47, XY, +13	30	Observation of anomalies in ultrasonography	Amniocentesis - 19	DWM, CCA, Ebstein anomaly, hyperechogenic large kidneys, polydactyly	Terminated
47, XY, +13	27	Observation of anomalies in ultrasonography	Cordocentesis - 28	DWM, Hypoplastic left heart	Terminated
47, XY, +18	42	Observation of anomalies in ultrasonography	Cordocentesis - 26	DWM, Hypoplastic left heart, ventricular septal defect	Terminated

DWM: Dandy-Walker malformasyonu, **CCA:** Corpus callozum agenesis

Conclusion

Prenatal diagnosis should be applied to all cases which are found anomaly in ultrasonography. Detailed ultrasonography should be performed in next weeks to patients who are diagnosed low risk in triple test result and determinant/determinants of chromosome anomaly should be searched.

References

1. Steele MW, Breg WR. Chromosome analysis of human amniotic fluid cells. *Lancet* 1966; 1: 383-6.
2. NICHD National Registry for Amniocentesis Study Group. Midtrimester amniocentesis for prenatal diagnosis: safety and accuracy. *JAMA* 1976; 236: 1471-6.
3. Simpson NE, Dallaire L, Miller JR, Siminovich L, Hamerton JL, Miller J. Prenatal diagnosis of genetic disease in Canada: report of a collaborative study. *Can Med Assoc J* 1976; 115: 739-48.
4. Tabor A, Philip J, Madsen M, Bang J, Obel EB, Norgaard-Petersen B. Randomized controlled trial of genetic amniocentesis in 4606 low-risk women. *Lancet* 1986; 1:1287-93.
5. Hobbins J, Grannum PA, Romero R, Reece EA, Mahoney MJ. Percutaneous umbilical blood sampling. *Am J Obstet Gynecol* 1985; 152: 1-6.
6. Nicolaides KH, Soothill PW, Rodeck CH, Campbell S. Ultrasound guided sampling of umbilical cord and placental blood to assess fetal well-being. *Lancet* 1986; 1: 1065-7.
7. Daffos F, Capella-Pavlovsky M, Forestier F. Fetal blood sampling during pregnancy with use of a needle guided by ultrasound: A study of 606 consecutive cases. *Am J Obstet Gynecol* 1985; 153: 655-60.
8. Maxwell DJ, Johnson P, Hurley P. Fetal blood sampling and pregnancy loss in relation to indication. *Br J Obstet Gynaecol* 1991; 98: 892-7.
9. Drugan A, Johnson MP, Evans MI. Amniocentesis. In: Evans MI (ed). Reproductive risks and prenatal diagnosis. connecticut, Appleton Lange, 1992: 191-200.
10. Şen C. Amniyosentez ve koryon villus örneklemesi. *Perinatoloji Dergisi* 2002; 2: 55-8.
11. Altınyurt S. Koryon villus örneklemesi. Amniyosentez ve kordosentez. *Türkiye Klinikleri Jinekoloji Obstetrik* 2002; 4: 303-5.
12. Cengizoğlu B, Karageyim Y, Kars B, Altundağ M, Turan C, Ünal O. Üç yıllık dönemdeki amniyosentez sonuçları. *Perinatoloji Dergisi* 2002; 1: 14-7.
13. Yayla M, Bayhan G, Yalınkaya A, Alp N. Amniyosentez ve kordosentez ile fetal karyotip tayini: 250 olguda sonuçlar. *Perinatoloji Dergisi* 1999; 7: 255-8.
14. Başaran S, Karaman B, Aydınlı K, Yüksel A. Amniyotik sıvı, trofoblast dokusu ve fetal kan örneğinde sitogenetik incelemeler: 527 olguluk seri sonuçları. *Jinekolojik Obstetrik Dergisi* 1992; 6: 81-9.
15. Yazıcıoğlu H.F, Dülger Ö, Çankaya A, Özyurt N, Aygün M, Çebi Z, ve ark. Süleymaniye Doğumevindeki prenatal invasif girişimlerin komplikasyon hızı, verim ve maliyet açısından analizi. *Perinatoloji Dergisi* 2004; 3: 128-34.

16. Dallaire L, Michaud J, Melankon SB, Potier M, Lambert M. Prenatal diagnosis of fetal anomalies during the second trimester of pregnancy. Their characterization and delination of defects in pregnancies at risk. *Prenat Diagn* 1991; 11: 629-35.

17. Ross HL, Elias S. Maternal serum screening for fetal genetic disorders. *Obstet Gynecol Clin North Am* 1997; 24: 33-47.

18. Yagel S, Anteby Ey, Hochner-Celnikier D, Ariel I, Chaap T, Neriah ZB. The role of midtrimester targeted fetal organ screening combined with the triple test and maternal age in the diagnosis of trisomy 21: A retrospective study. *Am J Obstet Gynecol* 1998; 178: 40-5.

19. Bahado-Singh R, Shahabi S, Karaca M, Mahoney MJ, Cole L, Oz UA. The comprehensive midtrimester test: high-sensitivity Down syndrome test. *Am J Obstet Gynecol* 2002; 186: 803-8.

20. Taner CE, Altınbaşoğlu FH, Özkirişçi FS, İmren A, Büyüktosun C, Özgenç Y, Derin G. İleri maternal yaş gebeliklerinde amniyosentez sonuçları. *Perinatoloji Dergisi* 2002; 4: 336-9.