Pregnancy Outcomes After Second Trimester Genetic Amniocentesis: Evaluation of 1070 Cases

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Abstract

Objective: To evaluate the distribution of indications, the success of the interventions and cell culture, and feto - maternal complications in second trimester karyotype aimed amniocentesis.

Methods: 1070 cases that amniocentesis was performed in our clinic between January 2002 - December 2005 were evaluated from the aspect of indication of interventions, karyotype results, fetal loss after intervention, success of cell culture, time and method of delivery, neonatal findings and neonatal outcomes.

Results: The most common indication was recognized as advanced maternal age (49.7%). In 1069 of our cases, except one, cells were able to grown in culture (99.9%). The rate of chromosome abnormality according to cell cultures were 3.9%. seven (0.7%) cases of fetal loss was observed after amniocentesis. Mean gestational age at delivery was 38.45±1.91 and mean birth weight was 3288.70±528.64 in cases amniocentesis was performed.

Conclusion: Amniocentesis is the most common prenatl diagnostic method after 15 gestational week. Advanced maternal age and increased risk in triple test is the most common reason for appication. The complication rate of amniocentesis for the mother and fetus is found low.

Keywords: Amniocentesis, indication, complication, success.

İkinci trimester genetik amniyosentez sonrası gebelik sonuçları: 1070 olgunun değerlendirmesi

Amaç: İkinci trimesterde yapılan karyotip amaçlı amniyosentez uygulanan olgularda endikasyonların dağılımını, girişim ve hücre kültür başarısını, feto–maternal komplikasyonları incelemek.

Yöntem: Ocak 2002 - Aralık 2005 tarihleri arasında kliniğimizde çeşitli endikasyonlarla amniyosentez uygulanan 1070 olgu retrospektif olarak girişim endikasyonları, karyotip sonucu, girişim sonrası fetal kayıp, hücre kültürü başarısı, doğum zamanı ve doğum şekli, yenidoğan bulguları ve neonatal prognoz yönünden değerlendirildi.

Bulgular: En sık endikasyon ileri anne yaşı olarak tespit edildi (%49.7). Olgularımızdan biri dışında 1069'ünde kültürde hücre üretildi (%99.9). Kültür sonuçlarına göre kromozom anomalisi oranı %3.9 olarak tespit edildi. Amniyosentez sonrası 7 (%0.7) olguda fetal kayıp meydana geldi. Amniyosentez uygulanan olguların ortalama doğum haftası 38.45±1.91 ve ortalama doğum ağırlığı 3288.70±528.64 olarak bulundu.

Sonuç: 15. gebelik haftasından sonra amniyosentez en sık kullanılan prenatal tanı yöntemidir. İleri anne yaşı ve üçlü testte artmış risk en sık uygulama nedenidir. Amniyosentez, anne ve fetus için komplikasyon oranı düşük bulunmuştur.

Anahtar Sözcükler: Amniyosentez, endikasyon, komplikasyon, başarı.

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Introduction

Amniocentesis based on examination of amniotic cells continues to being a significant invasive technique on prenatal diagnosis. It was first applied in 1950 for the purpose of sex determination.[1] Karyotype determination started as a classical way by culturing cells poured down to amnion fluid from skin and excretion system of fetus by Steele and Breg.[2] Today, main application indications are abnormality, advanced maternal age in scanning tests applied for trisomies, structural anomalies, chromosome anomaly birth case in ultrasonography and chromosome translocation known as in one of the couple.

Amniocentesis is traditionally applied in between 16th and 20th gestational weeks for karyotype determination. Rate of living cells is higher than non-living cells (>20th gestational week) in amnion fluid in this period.[3] If it is applied in early gestational weeks, rate of fetal loss is high.

Our purpose in this work is to evaluate indications of genetic amniocentesis interferences applied in our clinic and dispersion of chromosomal anomalies as to indications, fetal and neonatal results.

Methods

1070 cases which were applied amniocentesis due to the fact that they had high risk for chromosomal anomaly in between January 2002 – December 2005 were evaluated in terms of interference indications, fetal prognosis, genetic and neonatal results.

Genetic consultant was advised to all cases and genetic consultant was provided in a university hospital for couples requested consultant. Written and oral information were given about process technique and possible complications before interference. Couples were not directed for the decision. A detailed consent form was taken from couples who accepted the interference before application. Each fetus was examined in detail by ultrasonography before amniocentesis. Place of placenta, amount of amnion fluid and location to be done interference were established. Completed age 35 was determined as advanced maternity age; but it was not interpreted on its own as an amniocentesis indication. It was stated that risk evaluation would be done by non-invasive methods (triple test, detailed ultrasonography) to cases having or not having genetic consultant. Cut-off value was taken as 1/250 for increased risk in triple test; but amniocentesis option was suggested to couples by making risk evaluation in cases established as indicator for chromosome anomaly in ultrasonography in between cases with risk under 1/250. All cases were invited for control with karyotype result. Fetal losses after interference, birth date and birth kind, newborn diagnosis and neonatal prognosis were recorded in cases being followed up to the birth after process. Losses in between two weeks following interference were deemed as loss related to interference. In cases which could not being followed were phoned in order to learn birth week and prognosis.

Amniocentesis process was performed in between 16th and 22nd gestational weeks by four experienced and different operators working prenatal diagnosis and treatment unit. Skin cleaning was done by povidone-iodine. 2 ml, 5 ml or 10 ml injectors and 9 cm 20 or 22G spinal needles were used for puncture and aspiration. Interferences were done by free hand technique with accompany of ultrasonography (Siemens Sonoline G-50, Japan 2.5 – 5 MHzt convex probe).

It was paid attention puncturing generally to top and middle and even close areas of uterus and also not passing from placenta; but in obligatory cases to pass from placenta, entrance location of umbilical veins to placenta and edge areas were not used and cotyledon surface was passed as perpendicular. It was paid attention not existing fetal part and cordon segment in appropriate fluid pocked. Coming fluid was aspired by applying light negative pressure and amnion fluid was taken as 1 ml for each gestational week. The fluid was aspired by two different uterus entrances after determining localization of twins by ultrasonography in twin pregnancies. Routine local anesthesia during amniocentesis process and routine antibiotic prophylaxis after interference were not applied. 300 microgram anti – D IgG was applied to cases having Rh incompatibility.

Taken fluids were sent one of two different genetic laboratories (laboratories of Private hospi-

tal and university hospital), Giemsa taping technique was used. 25-50 metaphase plates deemed as enough for each case were evaluated in terms of numerical and structural disorder. Results were taken about in 21 days.

Statistics were formed by using MedCalc version 8.2 for Windows.

Results

Amniocentesis was applied to totally 1070 cases in between January 2002 – December 2005 for genetic purpose. Enough amnion fluids were obtained in all cases. Age and gestational weeks in cases which were applied amniocentesis were found respectively as about 32.83±6.23 and 18.25±1.41. Secondary puncture was applied in totally 18 cases (1.7%) due to twin gestation in 17 cases and due to maternal obesity in one case; and no production was occurred in cell culture due to cell failure at amnion fluid in 1 case done amniocentesis. Cordocentesis was offered to couple but they did not accept. In 39th week, the case gave a birth which was healthy and had normal xxx qualities weighing 3000 gr. Success of obtaining cell culture was 99.9% and culture failure was found as 0.1% in our amniocentesis series. No cordocentesis was applied to any case after amniocentesis (Table 1).

Dispersion as to indications of cases applied amniocentesis is shown in Table 2. Advanced maternity age within these indications constitutes the biggest group with 532 cases (49.7%). Increased risk in triple test with 318 cases (29.7%) and cases found fetal anomaly in ultrasonography with 108 cases (10.1%) followed this group. Main anomalies found in ultrasonography are shown in Table 3. Chromosome anomaly was found in 14 (13%) of 108 cases done due to fetal anomaly. These chromosome anomalies were Trisomy 21 in 5 cases, Trisomy 18 in 4 cases, Turner syndrome in 3 cases, triploidy in one case and Jumping translocation in one case. Ultrasonography anomalies seen in Trisomy 21 cases were found as early growing retardation, ventriculomegaly, early acid, cystic hygroma and hyperechogenic intestine. Holoprosencephaly, multiple anomaly (pleural effusion, strawberry head, choroid plexus cyst and brachycephaly) and cardiac anomalies were observed in 4 Trisomy 18 cases. Cystic hygroma was found in 3 45;X0 cases. Early growing retardation and ventriculomegaly in triploidy case and cardiac anomaly in jumping translocation case were found.

When cases being applied amniocentesis were evaluated as to entering method to uterine cavity, it was found that interferences were done as transamniotic for 864 cases (80.7%) and as transplacen-

Table 1. Demographic qualities of cases done amniocentesis.

	Average (±SD)
Age	32,83 ± 6,23
Gestation week during amniocentesis	18,25 ± 1,41
Gestation week during termination	21,08 ± 2,27
Gestation week at delivery	38,45 ± 1,91
Birth weight	3288,70 ± 528,64

Table 2. Dispersion of cases as to amniocentesis indications.

Amniocentesis indications	n (1070)	%
Advanced maternity age (age ≥35)	532	49,7
Increased risk at triple test	318	29,7
Fetal anomaly at USG	108	10,1
Increased risk at double test	32	3
NT increase	11	1,1
Maternal anxiety	12	1,1
Birth case with chromosome anomaly	40	3,8
Birth case with anomaly	7	0,7
Other	10	1

(Other: Maternal/paternal translocation, duchenne Type SMA, birth case with cystic fibrous, thalassemia carriage, adrenoleucodistrophy in first child)

Table 3. Main anomalies found in ultrasonography.

	n=108
Central nervous system anomaly	21 (%19)
Cardiac anomaly	17 (%16)
Choroid plexus cyst	13 (%12)
Cystic hygroma	13 (%12)
Hyperechogenic intestine	7 (%7)
Pelviecstasy	6 (%5)
Hydrops fetalis	6 (%5)
Omphalocele	5 (%5)
Hernia of diaphragm	4 (%4)
Echogenic intracardiac focus	2 (%2)
Other	14 (%13)

(Other: early growing retardation, urinary system anomalies, multiple anomalies, single umbilical artery, extremity anomalies and partial mol.)

tal for 207 cases (19.3%). When gestational complications were evaluated as to entering method to uterine cavity; 5 cases with abortus were found in transamniotic case group and 2 cases with abortus were found in transplacental case group. When complications were compared as to application of amniocentesis as if transplacental or transamniotic; there were no statistical difference between (abortus, early birth risk, early membrane rupture and intrauterine fetal loss) groups (p=0.62).

Chromosome anomaly rate as to amnion culture results were found as 3.9% in 1070 pregnants and as 3.9% in 1087 fetuses. Dispersion of cases found chromosome anomaly was as following: 23 trisomy 21, 4 trisomy 18, 6 Turner syndromes, 1 47, XXX, 1 triploidy and various hereditary or de novo structural chromosome anomalies. No chromosome anomaly was found in any case applied amniocentesis due to maternal anxiety. Fetal karyotype of 1027 pregnants and 1043 fetuses were found as normal. One case found trisomy 21 in amniocentesis decided to continue pregnancy and gave a birth at 39th week by vaginal way. Fetal karyotype results as to amniocentesis indications are shown in Table 3 (Table 4).

Rate of early fetal loss after amniocentesis was found as 0.7% in 1070 cases and as 0.6% in 1087 interferences. Amniocentesis was applied to two cases with advanced maternity age which came as a result of normal fetal karyotype and which did abort within one week after the process. Totally 3 cases miscarried within one week that partial mol was found in 1 case, oligohydroamnios and hyperechogenic intestine were found in 1 case and omphalocele and polyhydroamnios were found in 1 case in ultrasonography. Karyotype result was found as normal in these three cases. Pregnancy was terminated in 61 (5.7%) cases of 1071 amniocentesis cases. While the termination reason of 34 of 61cases was chromosomal anomaly, the indication of 27 cases was fetal anomalies found in ultrasonography against normal karyotype. Termination indications of 27 cases with normal karyotype were as following: Central nervous system anomaly in 13 cases, early growing retardation in 2 cases, skeleton dysplasia in 1 case, cardiac anomaly and hydrops fetalis in 7 cases, OIES complex (omphalocele, Cloacal exstrophy, anal atresia and spinal anomaly) in 1 case, adrenoleucodistrophy in 1 case, hernia of diaphragm in 1 case and

Table 4. Cytogenetics results as to amniocentesis indications.

Results	No	Amniocentesis Indications						
		Advanced maternity age (n=532)	With fetal anomaly in USG (n=108)	Increased NT (n=11)	Chromosome anomaly birth case (n=40)	Increased risk in triple test (n=318)	Increased risk in double test (n=32)	Other (n=29)
No production	1	1	-	-	-			
Culture success	1086							
Normal Karyotype	1043	526	102	8	41	311	32	19
Trisomy 21	23	10	5	1	-	4	-	2
Trisomy 18	4	-	4	-	-	-	-	-
45,X (Turner Syndrome)	6	-	3	2	-	1	-	-
47,XXY	1	1	-	-	-	-	-	-
69,XXX	1	-	1	-	-	-	-	
47,XY,+mar	1	1	-	-	-	-	-	
46,XY,inv(9)(p11;q13)	1	-	-	-	-	1	-	-
Jumping translocastion	1	-	1	-	-	-	-	-
Balanced translocastion	2	-	-	-	-	1	-	1
46,XX,15p+	1	-	-	-	-	1	-	-
46,XX,t(4;16)(p12;q22)	1	-	-	-	-	-	-	1
46,XX,t(17;22)(p13;q11.1)	1	-	-	-	-	-	-	1

(Other; Maternal anxiety, birth case with anomaly, adrenoleucodistrophy in first child, Duchenne type SMA, thalassemia carriage, maternal/paternal translocation).

multiple anomaly in 1 case. Average gestation week of cases of which gestation were terminated was found as 21.08±2.27. 667 (62.4%) cases gave birth by vaginal way and 232 (21.7%) cases gave birth by cesarean and birth method of 102 cases (9.5%) could not found. Average birth week of cases was found as 38.45±1.91 and average birth weight was found as 3288±528.64. When cases were evaluated as to gestation weeks at delivery, the birth of 1 case was occurred under 28th gestational week by vaginal way. Totally 10 cases gave birth in between 28th and 32nd gestational weeks and all of them gave birth with various indications by cesarean. Neonatal mortality in 3 cases of these newborns. Premature complications were ascertained as a reason for mortality. Totally 19 cases gave birth by cesarean and 18 cases gave birth by vaginal way in between 32nd and 36th gestational weeks. Neonatal mortality did not occur in any case. Mortality occurred in newborn period of one of cases which had >36 gestational week. We could not find birth types and neonatal results of 102 cases..

Discussion

Amniocentesis which is known as the oldest prenatal diagnosis method is frequently applied in between 16th and 18th gestational weeks for genetic diagnosis. Application indications are mainly advanced maternal age, increased risk in triple test, and child case with chromosome anomaly or fetal anomaly ascertainment in ultrasonography. Advanced maternal age was found as the most frequently interference reason with 532 cases (49.7%). Advanced maternal age was found as the most frequently interference reason in various amniocentesis series published in our country.[4,5,6]

Chromosomal anomaly was found in totally 43 cases (3.9%) in our amniocentesis series. This rate was ascertained as 3.3 – 4.5% in series published in our country.[4,5,6]

Chromosomal anomaly was found in 12 (2%) cases of 532 cases which was applied amniocentesis due to advanced maternal age. Sjögren et al[8] were reported this rate as 2.2% above age 35. Chromosomal anomaly rates were found as 1.2 – 13.3% in amniocentesis cases done by advanced maternal age indication.[6,9,10]

Chromosomal anomaly was found in 8 (2.5%) cases of 318 cases that we applied amniocentesis due to increased risk at triple test (≥1/250). Four (1.2%) of chromosome anomalies we found were Trisomy 21 and 1 case was Turner syndrome. Yüce et al found this rate as 3.7% in cases done due to increased risk at triple test.

There are significant differences between ascertainment rates of chromosomal anomaly in series which were applied amniocentesis due to fetal anomaly ascertainment in ultrasonography. This rate is reported between 4% and 27.1% in various series.[6,11,12,13] Chromosomal anomaly was found in 14 (12.9%) cases of 108 cases that we applied amniocentesis due to fetal anomaly ascertainment in ultrasonography. Probability of finding chromosome anomaly in amniocentesis by these data (rather than maternal age and triple test) increases with the existence of most frequent fetal anomaly.

Rates of fetal loss related to amniocentesis are not more than 0.5-1% by experienced people. Eddlemann et al found fetal loss rate as 0.15% in their series of 1605[14] cases. Armstrong et al[15] reported fetal loss rate as 0.2% in their series of 28163 cases. Loss rates are reported between 0.6% and 3.3% in series done in our country.[4,7,9] This rate was 0.7% in our series and it was compatible with the literature.

Cytogenetic analyze of amniotic cells shows fetal genotype by accuracy degree about 99%. We had to work with laboratories which agreed with related social security association due to the fact that there is no genetic laboratory in our association. Only 1 case did not have fetal cell production from the results came from laboratory that we sent amniotic fluids to and culture success was observed as 99.9%. We decided that non-production in that case resulted from the contamination of related laboratory. Güven et al[16] found that rate as 98% as our clinic. Müngen et al[17], found the rate of culture success as 98.2% in their series of 2068 cases published in 2006.

Conclusion

Amniocentesis is the most used after 15th gestational week antenatal diagnosis technique having minimal complications. Though the reason of the most usage is determination of fetal karyotype, it may be applied with many indications. The most important disadvantage of it is to obtain the results later than other prenatal diagnosis methods; but later results may be obtained by development of the technology and application of methods such as FISH and PCR. Advanced maternal age and increased risk at triple test took place in our amniocentesis indications. We found chromosome anomaly rate as 3.9% and rate of fetal loss as 0.7% after amniocentesis. These results are parallel to literature.

References

1. Drugan A, Johnson MP, Evans MJ. Amniocentesis. In: Evans MI (Ed). Reproductive risks and prenatal diagnosis. Connecticut Appleton & Lange. 1992: 191-200.
2. Steele WW, Breg WR. Chromosome analysis of human amniotic fluid celss. *Lancet* 1966; 1: 383-5.
3. Gerbie AB, Elias S. Technigue for midtrimester amniocentesis for prenatal diagnosis. *Semin Perinatol* 1980; 4: 159.
4. Yayla M, Bayhan G, Yalınkaya A, Alp N. Yüksek riskli gebeliklerde 2. trimester genetik amniyosentez: 165 olgunun klinik değerlendirilmesi. *Perinatoloji Dergisi* 1999; 7: 40 – 46.
5. Cengizoğlu B, Karageyim Y, Kars B, Altundağ M, Turan C, Ünal O. Üç yıllık dönemdeki sonuçları. *Perinatoloji Dergisi* 2002; 1: 14 – 7.
6. Yüce H, Çelik H, Gürateş B, Erol D, Hanay F, Elyas H. Karyotip analizi amacı ile genetik amniyosentez uygulanan 356 olgunun retrospektif analizi. *Perinatoloji Dergisi* 2006; 14: 73-6.
7. Başaran S, Karaman B, Aydınlı K, Yüksel A. Amniyotik sıvı, trofoblast dokusu ve fetal kan örneğinde sitogenetik incelemeler. 527 olguluk seri sonuçları. *Jinekoloji Obstetrik Dergisi* 1992; 6: 81-9.
8. Sjögren B, Uddenberg N. Decision making duruing prenatal diagnostic procedure. A questionnaire and interview Study of 211 women participating in prenatal diagnosis. *Prenat Diagn* 1989; 9: 263-73.
9. Bal F, Uğur G, Yıldız A, Şahin İ, Menekşe A. 2. trimester riskli gebeliklerinde amniyosentez uygulamaları. *Türkiye Klinikleri Jinekoloji Obstetrik Dergisi* 1995; 5: 249-56.
10. Taner CE, Altınbaşoğlu FH, Özkirişçi FH, İmren A, Büyüktosun C, Özgenç Y, Derin G. İleri maternal yaş gebeliklerinde amniyosentez sonuçları. *Perinatoloji Dergisi* 2002; 4: 336-9.
11. Rizzo N, Pittalis MC, Pilu G, Orsini LF, Perolo A, Bovicelli L. Prenatal karyotyping in malformed fetus. *Prenat Diagn* 1990; 10: 17-23.
12. Dallaire L, Michaud J, Melancon SB, Potier M, Lambert M, Mitchell G, Boisvert J. Prenatal diagnosis of fetal anomalies during the second trimester of pregnancy: their characterization and delineation of defects in pregnancies at risk. *Prenat Diagn* 1991; 11: 629-35.
13. Stoll C, Dott B, Alembik Y, Roth M. Evalution of routine prenatal ultrasound examination in detecting fetal chromosomal abnormalities in a low risk population. *Hum Genet* 1993; 91: 37-41.
14. Eddleman K, Berkowitz R, Kharbutli Y, et al. Pregnancy loss rates after midtrimester amniocentesis: The FASTER trial. *Am J Obstet Gynecol* 2003; 189: 111.
15. Armstrong J, Cohen AW, Bombard AT, et al. Comparison of amniocentesis – related loss rates between obstetrician-gynecologists and perinatologists. *Obstet Gynecol* 2002; 99: 65.
16. Güven MA, Ceylaner S. Amniyosentez ve kordosentez ile prenaral tanı: 181 olgunun değerlendirilmesi. *Perinatoloji Dergisi* 2005; 13: 25-9.
17. Müngen E, Tütüncü L, Muhcu M, Yergök YZ. Pregnancy outcome following second-trimester amniocentesis: a case-control study. *Am J Perinatol* 2006; 23: 25-30.