DOI: 10.5350/SEMB.20160103093300

Genetic Amniocentesis Results: Analysis of the 3721 Cases

Ali Acar[1], Fedi Ercan[1], Selman Yildirim[2], Huseyin Gorkemli[1], Kazim Gezginc[1], Osman Balci[1], Harun Toy[1], Aysegul Zamani[2], Sevcan Sarikaya[1], Berkan Sayal[1], Metin Capar[3], Mehmet Cengiz Colakoglu[1]

ABSTRACT:

Genetic amniocentesis results: analysis of the 3721 cases

Objective: To retrospectively investigate the 7-year experience of prenatal diagnosis of fetal chromosome aberrations by second-trimester genetic amniocentesis.

Material and Method: Data were collected at Meram Medical Faculty Obstetric and Gynecology Department between January 2007 and January 2014 from cytogenetic analyses of cultured amniocytes from second-trimester amniocentesis. The main indications for amniocentesis included advanced maternal age, abnormal maternal serum screening results, and abnormal ultrasound findings. Chromosome aberrations included autosomal aneuploidies, sex chromosome aneuploidies, polyploidies, and rearrangements.

Results: A total of 3702 amniocenteses were performed and analyzed for chromosome aberrations. Among these, 1677 (45.1%) were for abnormal maternal serum screening results, 1332 (35.8%) for advanced maternal age, 586 (15.8%) for abnormal ultrasound findings, and 126 (3.3%) for other reasons. Chromosome aberrations were detected in 131 (3.6%) cases, including fetuses of 53 older mothers, 37 mothers with abnormal serum screening results, 34 mothers with abnormal ultrasound findings, and 7 mothers with other reasons for amniocentesis. Of fetuses with chromosome aberrations, 106 (80.9%) had numerical chromosomal disorder. The other 25 (19.1%) cases included structural chromosomal disorder.

Conclusions: For daily practice, our data could offer a database for proper genetic counseling, such as termination issues and future pregnancies.

Keywords: Amniocentesis, chromosome aberration, prenatal diagnosis

ÖZET:

Genetik amniyosentez sonuçlarımız: 3721 vakanın analizi

Amaç: İkinci trimester genetik amniyosentez ile fetal kromozomal anomalileri saptamada 7 yıllık prenatal tanı verilerinin retrospektif olarak analizi.

Gereç ve Yöntem: Veriler Meram Tıp Fakültesi Kadın Hastalıkları ve Doğum Anabilim Dalı'nda Ocak 2007 ile Ocak 2014 tarihleri arasında yapılan ikinci trimester genetik amniyosentezlerin amniyosit kültürü sitogenetik analizi verilerinden oluşturulmuştur. Amniyosentez için ana endikasyonlar ileri anne yaşı, anormal maternal serum taraması sonuçları ve anormal ultrason bulgularından oluşmaktadır. Kromozomal anomaliler; otozomal anöploidiler, seks kromozomu anöploidileri, poliploidiler ve yeniden düzenlenmeleri kapsamaktadır.

Bulgular: Kromozomal anomalilerin teşhisi için toplam 3721 amniyosentez yapıldı. Bunların 1677'si (%45.1) anormal maternal serum taraması sonuçları, 1332'si (%35.8) ileri anne yaşı, 586'sı (%15.8) anormal ultrason bulguları ve 126'sı da (%3.3) diğer nedenlerle yapıldı. Kromozomal anomali toplam 131 (%3.6) vakada tespit edildi. Bunların 53'ü ileri anne yaşı, 37'si anormal serum tarama testi sonucu, 34'ü anormal ultrason bulguları ve 7'si diğer nedenlerle amniyosentez yapılan vakalardı. Kromozomal anomali teşhis edilen hastaların 106'sında (%80.9) sayısal kromozomal anomali vardı. Diğer 25 (%19.1) hastada ise yapısal kromozomal anomali tespit edildi.

Sonuçlar: Verilerimiz terminasyon konuları ve sonraki gebelikler ile ilgili olarak günlük pratikte uygun genetik danışmanlık sağlanabilmesi için bir veritabanı sağlayabilir.

Anahtar kelimeler: Amniyosentez, kromozom aberasyon, prenatal tanı

Ş.E.E.A.H. Tıp Bülteni 2016;50(1):33-8

[1]Necmettin Erbakan University, Meram Medical Faculty, Department of Obstetrics and Gynecology, Konya - Turkey
[2]Necmettin Erbakan University, Meram Medical Faculty, Department of Medical Genetics, Konya - Turkey
[3]Ozel Medicana Konya Hospital, Clinic of Obstetrics and Gynecology, Konya - Turkey

Address reprint requests to / Yazışma Adresi:
Fedi Ercan,
Necmettin Erbakan University, Meram Medical Faculty, Department of Obstetrics and Gynecology, Konya - Turkey

E-mail / E-posta:
fediercan@gmail.com

Date of receipt / Geliş tarihi:
November 26, 2015 / 26 Kasım 2015

Date of acceptance / Kabul tarihi:
January 3, 2016 / 3 Ocak 2016

INTRODUCTION

Genetic amniocentesis procedure is the most common invasive diagnostic test, used to detect the chromosome aberrations in the fetus. Fetal karyotyping detected by medical cytogenetics is one of the most common areas that this application is used for. When a karyotype abnormality is detected, this allows the termination of the pregnancy or a more appropriate obstetric care for the future pregnancies (1,2). Amniocentesis is first performed to detect the fetal gender in 1956, with the presence of "Barr" body in the obtained fetal cells (3). Then in 1966, it was possible to determine the fetal karyotype in the amniotic fluid (4).

90% of the chromosome aberrations observed in the prenatal period are the numerical aberrations of 21st, 18th and 13th chromosomes and of sex chromosomes (X and Y chromosomes) (aneuploidy). Trisomy 21, trisomy 18 and trisomy 13 are the most common otosomoal trisomies. Turner's syndrome and Klinefelter's syndrome are the most common numerical sex chromosome aberrations (5).

In this study, the results of a 7-year data (January 2007-January 2014), including the genetic amniocentesis performed regarding the prenatal diagnosis of the chromosome aberrations in Necmettin Erbakan University, Meram Medical Faculty, Department of Obstetrics and Gynecology, are presented.

MATERIAL AND METHOD

The data of 3721 pregnant women who applied to Necmettin Erbakan University, Meram Medical Faculty, Department of Obstetrics and Gynecology and had genetic amniocentesis with various indications between January 2007 and January 2014 were evaluated retrospectively. The data was evaluated in terms of indication, complication, cell culture success and the genetic results.

The main indications for the genetic amniocentesis in our study include, advanced maternal age, abnormal maternal serum screening results and abnormal ultrasound findings and some causes examined under the title "other" (history of having a child with congenital abnomality, family history related with chromosome aberrations, abnormally elevated serum alpha-fetoprotein levels, maternal anxiety). Advanced maternal age is accepted as being over 35 years old at birth. Abnormal maternal serum screening test includes the pregnant women who carry high risk for trisomy 18 and 21 (measured combined risk for trisomy 21 is ≥1/270, and for trisomy 18 ≥1/100). Abnormal ultrasound findings are defined as the presence of one or more of the soft markers (increased nuchal fold thickness, choroid plexus cyst, pyelectasis, single umbilical artery, oligohydramnios in the early weeks, intrauterine growth retardation in the early weeks, polyhydramnios in the early weeks, hyperechogenic bowel, ventriculomegaly, echogenic intracardiac foci) which increase in fetal abnomalities and in chromosome aberration risk. The family history of chromosome aberrations include aneuploidy, translocations, inversions and marker chromosomes. The patients who had maternal anxiety, but having maternal serum screening test results below the detected threshold level (in the low-risk group), were named as "amniocentesis depending on the family's request" and kept under "other reasons" title.

In our clinic, informed consent is obtained before the intervention from all patients who accept the amniocentesis procedure. Before the intervention, all pregnant women were screened for Rh incompatibility and serological screening for Hepatitis B and C, of the viral hepatitis was performed. For the amniocentesis procedure, Voluson 730 Pro ultrasound device and 3.5 MHz transabdominal probe was used. The skin was cleaned with polyvidone iodine, and for puncture and aspiration, disposable 2 and 20 ml syringes and 20 or 22 G spinal needles were used. The interventions were performed with ultrasound-guided "free hand technique". The first aspirated amniotic fluid of 2 ml was spared in case of maternal contamination. Then by aspirating the fluid by applying a slight negative pressure, it was collected in a 20 ml syringe in the manner of 1 ml per week of gestation. Up to 3 attempts have been performed to obtain amniotic fluid. In 112 of a total of 3721 patients, 3 times were needed for the needle entry. Patients were observed in the clinic for 1 hour after the intervention. Each patient was prescribed oral antibiotics (Amoxicillin 875 mg for 3

days) and paracetamol.

The samples were sent to genetics lab, and after 15-20 days of cell culture, the samples which at least 20 metaphase images were obtained, were examined. The chromosomal aberrations (numerical or structural) include aneuploidy, polyploidy, mosaicism, deletion, duplication, inversion, balanced translocation, unbalanced translocation, ring chromosomes and marker chromosomes.

RESULTS

A total of 3721 genetic amniocentesis were performed in Necmettin Erbakan University, Meram Medical Faculty, Department of Obstetrics and Gynecology between January 2007 and January 2014. The mean gestational age of the pregnant women who had genetic amniocentesis 17.62±1.85 and their mean age was 32.11±6.67. The procedure was performed to 1677 (45.1%) patients for abnormal maternal serum screening, to 1332 (35.8%) for advanced maternal age and to 586 (15.8%) for abnormal ultrasound findings, and to 126 (3.3%) for other reasons (Table-1).

The chromosomal aberrations were detected in a total of 131 (3.6%) pregnant women. Of these, 53 had advanced maternal age, 37 had abnormal maternal serum screening, 34 had abnormal ultrasound findings and 7 had other reasons. The mean age of pregnant women with chromosomal aberrations was 32.05±5.95 (minimum: 17 years, maximum: 46 years).

Numerical chromosomal aberrations were detected in 106 (80.9%) of the cases with

Table-1: The distribution of chromosomal abnormalities and cell culture failures of 3721 patients who had genetic amniocentesis according to indications of procedure

Results	n	Amniocentesis Indications			
		Abnormal maternal serum screening	Advanced maternal age	Abnormal ultrasound findings	Other
Numerical Abnormalities	106 (80.9%)	28	40	32	6
Structural Abnormalities	25 (19.1%)	9	13	2	1
Total Chromosomal Abnormalities	131 (3.6%)	37	53	34	7
No proliferation	26 (0.7%)	4	6	12	4
Normal karyotype	3564 (95.7%)	1636	1273	540	115
Total	3721 (100%)	1677 (45.1%)	1332 (35.8%)	586 (15.8%)	126 (3.3%)

Table-2: Types of numerical abnormalities and their frequency in all abnormalities

Results	n
47,XY+21(Standard Down Syndrome)	35
47,XX+21 (Standard Down Syndrome)	28
47,XX+18 (Edwards Syndrome)	8
47,XY+18 (Edwards Syndrome)	4
45,X (Turner Syndrome)	6
69,XXX (Triploidy)	4
47,XY+13 (Patau Syndrome)	4
47,XX+13 (Patau Syndrome)	1
47,XXY (Klinefelter Syndrome)	2
47,XYY	1
47,XXX	1
47,XX+mar	3
46,XY[18]/47,XY+mar[1]/48,XY+mar+mar[1]	1
47,XXY[5]/46,XY[26]	1
46,XX[6]/47XX+mar[2]	1
45,X[30]/46,XY[9]	1
47,XXY[7]/46,XY[90] (7%)	1
47,XX+21[26]/46,XX[17] (Mozaic Down Syndrome)	1
47,XY+mar[1],46,XY(24)	1
46,XX[12]/47,XX+mar[38]	1
XY (72%), XXY (28%)	1
Total Numerical Abnormalities	106 (80.9%)

Table-3: The type of structural abnormalities and their frequency in all abnormalities

Results	n
46,XY,inv(6)	2
46,XY,inv(9)	2
46,XX,inv(9)	2
46,XY,t(2;12)(q23;q22)	1
46,XX,t(10q;18p)	1
46,XX,t(7q;15q)	1
46,XY,t(19q;22q)	1
46,XX[27]/47,XXX[23]	1
46,XY,t(6p23;13q12)	1
46,XX,t(10;13)(q11.2;q12)	1
46,XY,inv(6)(p23q21)	1
46,XX inv9(pat)	1
46,XY, t(12;16)(q24.1;q24)	1
46,XY,der(13,14)+13 (trizomi 13)	1
46,XX,t(2;10)(p25;q24)	1
46,XX, t(4q;11q)(q35;q21)	1
46,XY,t(3p14;6q25)	1
45,XX,t(13q;14q)	1
46,XY,del(9)(pter->p21)	1
46,XY,inv(8)(p23.1q11.22)	1
46,XX,t(1;12)(q32p13)pat	1
45,XX,rob(14;21)	1
Total	25 (19.1%)

chromosomal aberrations (Table-2). Of these, 63 was trisomy 21, 12 was trisomy 18, 5 was trisomy 13 and 10 was sex chromosome abnormalities. In other 25 (19.1%) cases, there was structural chromosome abnormalities (Table-3).

Amniotic fluid could be obtained in 82% (3051/3721) of cases in the first trial, in 15% (558/3721) cases in the second trial, and in 3% (112/3721) cases, in the third trial. In 26 (0.7%) of 3721 cases who had invasive intervention, the karyotyping couldn't be done due to previous bleeding and contamination. The success rate was found as 99.3%.

In 16 (0.42%) of the patients who had amniocentesis, amniotic fluid leakage which lasted for 24-48 hours after the intervention, was detected. In all of these patients, the pregnancy continued without any problem. In addition, pregnancy loss in 16 patients developed after amniocentesis. The pregnancy loss rate was observed as 0.42% (16/3721). In all patients who experienced pregnancy loss are the patients who had the amniotic fluid obtained with a single needle entry. Five patients admitted to the hospital for complaints of abdominal pain and nausea 2 days after the amniocentesis, and diagnosed with abortus incipiens, resulting in spontaneous abortion at the hospital after admission. The other 11 patients admitted to the hospital with symptoms of fever, chills, malodorous vaginal discharge and nausea in 10 days after the amniocentesis, and their pregnancies were terminated with the diagnosis of chorioamnionitis.

DISCUSSION

Genetic amniocentesis is the most common invasive procedure referred for the prenatal cytogenetic diagnosis. Although the most frequent indication of this process is defined as the advanced maternal age in several studies (6,7), in the recent studies, it is observed that this seems to undergo change into abnormal maternal serum screening (8,9). Also in our series, abnormal maternal serum secreening test was the reason to perform genetic amniocentesis to most of the pregnant women (1677/3721, 45.1%). This situation may be connected to chorionic villus sampling to not being able to be performed in every center, despite the increasing use of dual screening which is in rise in the recent years, and the forwarding of these patients to amniocentesis.

The frequency of diagnosis of chromosomal abnormalities in pregnant women who had genetic amniocentesis was reported to range between 1.9%-5.8% (10-13). The frequency of diagnosis of chromosomal abnormality in our study is 3.6%. Trisomy 21 is the most commonly diagnosed chromosomal abnormality (63/131, 48%), followed by trisomy 18 (12/131, 9.1%).

Advanced maternal age is known to increase the karyotype abnomality risk. In this study, the group with the most common chromosomal abnormality was the group who had amniocentesis for advanced maternal age. The most common indication was detected to be the advanced maternal age in 131 chromosomal abnormalities detected in our study (53/131, 40.45%). The mean age of the pregnant women who were detected to have chromosomal abnormalities was 32.05±5.95. In a study, trisomy 21 risk in pregnant women over 35 years of age was found as 2.2%, and in women over 40 years of age, as 5.3% (14). In our series, trisomy 21 risk was found as 3.1% in women over 35 years of age, and in women over 40, as 6.3%.

Amniocentesis is performed after the 15th gestational week. Before 15th week of gestation, it carries the risks of increased culture failure, pregnancy loss rates and amniotic fluid leakage (15). In our study, genetic amniocentesis was performed to all pregnant women after 15th gestational week.

During amniocentesis, even with one or two needle entries, there was statistically no significant differences of fetal loss rates, 3 or more entries were observed to increase the rates (16). In addition, in studies, the fetal loss rates were evaluated in transplacental entries and entries from other areas, and no significant difference was detected (17,18). No data was found regarding transplacental entry when our records were analyzed. Pregnancy loss was detected in 16 patients in our study (0.42%). In a study, the pregnancy loss rate in the second trimester was found as 1% (19).

Maternal mortality has been reported due to

E.coli septicemia within 48 hours after amniocentesis (20). Maternal mortality was not observed in our series. The other very rare complications seen in the mother with amniocentesis are perforation in the visceral organs, amniotic fluid embolism and Rh isoimmunisation (21).

There are studies that give the frequency of fetal chromosomal abnormality in fetuses with abnormalities detected at ultrasound between 6.8%-27.1% (22,23). In our seies we detected karyotype abnormality in 6.08% of the pregnant women with an abnormal ultrasound finding. This difference may result from the absence of detected soft markers in the abnormal ultrasound findings in some studies.

Prenatal invasive tests are often used to investigate the specific chromosomal abnormalities and the most commonly searched abnormality is trisomy 21. Today, the efforts are directed to effective prenatal screening programs that would reduce the number of amniocentesis, than to prenatal diagnosis of the chromosomal aberrations using amniocentesis.

In conclusion, we found the rate of chromosomal abnormality rate as 3.54% and fetal loss rate as 0.42% in our study. In addition, abnormal maternal serum screening results (45.1%) and the advanced maternal age (35.8%) are our most common amniocentesis indications. Among the limitations of our study are the lack of data stating the amniotic fluid leakage with transplacental needle entry and the amniocentesis morbidity other than abortions. Our data may provide a database for an appropriate genetic counseling regarding the termination issues and the future pregnancies, in daily practice.

Acknowledgement: We would like to express our sincere thanks and appreciation to Mrs. Nurcan Sağlam and Mrs. Naciye Öney, the responsible nurses of Necmettin Erbakan University, Meram Medical Faculty, Department of Obstetrics and Gynecology, Unit of Perinatology, for their rigorous and selfless acts in preparation and keeping records of the data.

REFERENCES

1. *Platt LD, DeVore GR, Lopez E, Herbert W, Falk R, Alfi O. Role of amniocentesis in ultrasound-detected fetal malformations. Obstet Gynecol 1986; 68: 153-5.*
2. *Rochelson BL, Trunca C, Monheit AG, Baker DA. The use of a rapid in situ technique for third-trimester diagnosis of trisomy 18. Am J Obstet Gynecol 1986; 155: 835-6.* **[CrossRef]**
3. *Fuchs F, Rus P. Antenatal sex determination. Nature 1956; 177: 330.* **[CrossRef]**
4. *Steele MW, Breg WR Jr. Chromosome analysis of human amniotic-fluid cells. Lancet 1966; 19: 383-5.* **[CrossRef]**
5. *Summers AM, Langlois S, Wyatt P, Wilson RD. Prenatal screening for fetal aneuploidy. Society of Obstetricians and Gynaecologists of Canada. J Obstet Gynaecol Can 2007; 29: 146-79.* **[CrossRef]**
6. *Blackwell SC, Abundis MG, Nehra PC. Five-year experince with midtrimester amniocentesis performed by a single group of obstetricians-gynecologists at a community hospital. Am J Obstet Gynecol 2002; 18: 1130-2.* **[CrossRef]**
7. *Marthin T, Liedgren S, Hammar M. Transplacental needle passage and other risk-faktors associated with second trimester amniocentesis. Acta Obstet Gynecol Scand 1997; 76: 728-32.* **[CrossRef]**
8. *Turhan-Öztürk N, Eren Ü, Seçkin NC. Second trimester genetic amniocentesis: 5 year experience. Arch Gynecol Obstet 2005; 271: 19-21.*
9. *Türkyılmaz A, Budak T. Laboratuvarımıza prenatal tanı için sevk edilen ailelerde endikasyon ve sonuç uygunluklarının değerlendirilmesi. Dicle Tıp Dergisi 2007; 34: 258-63.*
10. *Chaabouni H, Chaabouni M, Maazoul F, M'Rad R, Jemaa LB, Smaoui N, et al. Prenatal diagnosis of chromosome disorders in Tunisian population. Ann Genet 2001; 44: 99-104.* **[CrossRef]**
11. *Kong CW, Leung TN, Leung TY, Chan LW, Sahota DS, Fung TY, et al. Risk factors for procedure-related fetal losses after mid-trimester genetic amniocentesis. Prenat Diagn 2006; 26: 925-30.* **[CrossRef]**
12. *Timur A, Uyar İ, Gülhan İ, Tan Saz N, İleri A, Özeren M. Genetik amniyosentez yapılan 16-22 haftalık gebelerin amniyosentez sonuçlarının değerlendirilmesi. Perinatoloji Dergisi 2013; 21: 101-6.*
13. *Pazarbaşı A, Demirhan O, Taşdemir D, Tunç E, Özgünen FT, Alptekin D, et al. Amniyosentez ile tanı konulan 4707 olgunun sitogenetik bulgularının değerlendirilmesi. Cukurova Medical Journal 2011; 36: 8-14.*
14. *Sjögren B, Uddenberg N. Decision making during the prenatal diagnostic procedure. A questionnaire and interview study of 211 women partipating in prenatal diagnosis. Prenat Diagn 1989; 263-73.*
15. *Johnson JM, Wilson RD, Singer J, Winsor E, Harman C, Armson BA, et al. Technical factors in early amniocentesis predict adverse outcome. Results of the Canadian Early (EA) versus Mid-trimester (MA) Amniocentesis Trial. Prenat Diagn 1999; 19: 732-8.* **[CrossRef]**
16. *Bubb JA, Matthews AL. What is new in prenatal screening and diagnosis? Prim Care 2004; 31: 561-82.* **[CrossRef]**
17. *Tabor A, Madsen M, Obel E, Philip J, Bang J, Norgaard-Pedersen B. Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. Lancet 1986; 1: 1287-93.* **[CrossRef]**
18. *National Registry for amniocentesis study group: midtrimester amniocentesis for prenatal diagnosis: safety and accuracy. JAMA 1976; 236: 1471-6.* **[CrossRef]**

19. *Eddleman KA, Malone FD, Sullivan L, Dukes K, Berkowitz RL, Kharbutli Y. Pregnancy loss rates after midtrimester amniocentesis. Obstet Gynaecol 2006; 108: 1067-72.* **[CrossRef]**
20. *Thorp JA, Helfgott AW, King EA, King AA, Minyard AN. Maternal death after second-trimester genetic amniocentesis. Obstet Gynecol 2005; 105: 1213-5.* **[CrossRef]**
21. *Dodgson J, Martin J, Boswell J, Goodall HB, Smith R. Probable amniotic fluid embolism precipitated by amniocentesis and treated by exchange transfusion. Br Med J 1987; 23; 294: 1322-3.* **[CrossRef]**
22. *Rizzo N, Pittalis MC, Pilu G, Orsini LF, Perolo A, Bovicelli L. Prenatal karyotyping in malformed fetuses. Prenat Diagn 1990; 10: 17-23.* **[CrossRef]**
23. *Dallaire L, Michaud J, Melancon SB, Potier M, Lambert M, Mitchell G, et al. 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.* **[CrossRef]**