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Amniocentesis results of Manisa tertiary care in 2012

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Abstract

Objective: The aim of this study was to evaluate the results of invasive amniocentesis procedures performed for karyotype analyzing in our clinic in 2012.

Methods: The data of 83 cases, to whom performed amniocentesis for karyotype analyzing by reason of first trimester screening test's increased risk (≥1/270), second trimester screening test's increased risk (≥1/270), determination of abnormality with ultrasound and other causes, was analyzed retrospectively in 2012.

Results: Eighty of 83 amniocentesis procedures performed were successful in tissue culture. Culture success rate in amniocentesis was determined as 96.4%. Chromosomal abnormality was determined as 10% of these cases (8/80). The most common indication of amniocentesis was second trimester screening test's increased risk. Amniocentesis was performed to 30 cases for second trimester screening test's increased risk and chromosomal abnormality was determined in two cases (6.6%). The second indication for amniocentesis was increased risk of first trimester screening test which was 34.9% (29/83). Chromosomal abnormality was found in 13.8% of these cases (4/29). The other indications were the determination of abnormality during ultrasonography as 15.6%, family request as 9.6%, increased quadruple screening test as 2.4%, and history of delivery with chromosomal abnormality as 2.4%. No complication was seen after amniocentesis in all 83 cases.

Conclusion: Amniocentesis is the most applicable, with the least complication and the oldest prenatal diagnosis procedure in practice. Although chorionic villus sampling is first diagnosis test after first trimester screening test practically, pregnant women could come to reference centers like our hospital for different reasons after 14 weeks of gestation. Therefore, increased risk of first trimester screening test is determined as a high rate indication for amniocentesis in our center.

Key words: Amniocentesis, karyotype analysis, screening test in pregnancy.

Manisa ili üçüncü basamak 2012 yılı amniyosentez sonuçları

Amaç: Bu çalışmada, 2012 yılında kliniğimizde karyotip tayini amaçlı yapılan girişimsel işlemlerden amniyosentezlere ait sonuçların değerlendirilmesi amaçlanmıştır.

Yöntem: 2012 yılında ilk trimester tarama testinde yüksek risk (≥1/270), ikinci trimester tarama testinde yüksek risk (≥1/270), ultrasonografide anomali izlenmesi ve diğer sebeplerle uygulanan karyotip tayini amaçlı amniyosentez yapılan 83 olgunun verileri retrospektif olarak değerlendirildi.

Bulgular: Gerçekleştirilen 83 amniyosentez girişiminden, 80'inde doku kültürü başarılı oldu. Amniyosentezde kültürde başarı oranı %96.4 olarak tespit edildi. Üreme tespit edilen olgularda %10 oranında kromozom anomalisi tespit edildi (8/80). Endikasyon olarak en büyük dilimi, ikinci trimester tarama testinde yüksek risk çıkan grup oluşturdu. İkinci trimester tarama testinde yüksek risk tespit edilen 30 (%36.1) olguya, karyotip tayini amaçlı amniyosentez uygulandı ve 2 (%6.6) olguda kromozom anomalisi izlendi. Bu endikasyonu; %34.9 ile ilk trimester tarama testinde artmış risk izledi (29/83). Bu olgularda da %13.8 oranında kromozom anomalisi izlendi (4/29). Diğer endikasyonlar ise %15.6 ultrasonografide anomali saptanması, %9.6 aile isteği, %2.4 artmış dörtlü test riski, %2.4 de daha önce kromozom anomalili bebek doğurma öyküsü idi. Amniyosentez sonrası 83 olgunun hiçbirinde komplikasyon yaşanmadı.

Sonuç: Amniyosentez, pratikte en sık uygulanan, komplikasyonu en az olan ve bilinen en eski prenatal tanı yöntemidir. Pratik olarak ilk trimester tarama testi sonrası, birincil tanı yöntemi koryon villus örneklemesi olmasına rağmen, hastanemiz gibi referans merkezlerine farklı nedenlerle gebeler 14. gebelik haftasından sonra gelebilmektedir. Bu nedenle ilk trimester tarama testi risk yüksekliği, merkezimizde amniyosentez endikasyonu olarak yüksek oranda saptanmıştır.

Anahtar sözcükler: Amniyosentez, gebelik tarama testleri, karyotip analizi.

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Introduction

By means of the invasive procedures used for prenatal diagnosis, it has become possible to get information about fetal karyotype. Amniocentesis was first carried out for the determination of gender cells in 1950s.[1] In 1966, by culturing the cells flowing into amniotic fluid from fetal skin and gastrointestinal systems, it was initiated to do karyotype analysis in real terms.[2] Today, it is widely accepted as the method most easily performed, with the least maternal and fetal morbidity risk among prenatal invasive diagnostic methods if especially carried out at second trimester.[3]

During the last 35 years, the most frequent indication for amniocentesis has been due to the fetal karyotype analysis for advanced maternal age. In addition, amniocentesis is used for the diagnosis of diseases associated with DNA analysis (hematologic diseases associated with hemoglobin), enzyme analysis determination associated with metabolic diseases and determination of congenital infections by PCR (polymerase chain reaction).

Common use of screening tests and prevalent examination by ultrasonography for the diagnosis of chromosomal abnormalities recently has caused the demand on amniocentesis to increase. Amniocentesis for karyotyping purposes can be carried out beginning from 10 weeks of gestation; however, loss rate in these early periods is higher than those carried out in the second trimester. If it is carried out after 20 weeks of gestation, the period to get results increases due to the difficulty of reproduction in the culture. Therefore, amniocentesis for genetic purposes is applied between 16 and 20 weeks of gestation.[4]

Our aim in this study was to evaluate feto-maternal complications, distribution of amniocentesis indications, and our success of obtaining culture after amniocentesis procedures for fetal karyotyping applied within last year at our university serving as a tertiary care unit in Manisa.

Methods

Eighty-three pregnants who undergone karyotype analysis by amniocentesis for prenatal diagnosis were evaluated retrospectively in Perinatology Department, Medicine Faculty, Celal Bayar University in 2012 in terms of invasive indications, fetal prognosis, cell culture success, and genetic results. As a standard procedure, all cases and their husbands were informed verbally before the procedure about the procedure technique and possible complications. Before the procedure, written informed consent forms were received from couples who accepted the initiative. All pregnant women were evaluated in terms of Rh incompatibility. Voluson 730 (General Electric Healthcare, Milwaukee, WI, USA) 3.5 MHz transabdominal probe was used in the initiatives. Following the systematic and detailed ultrasonography evaluation and placenta localization, 1-2 ml amniotic fluid was taken and discarded into a separate injector by 20 G needle at 16-20 weeks of gestation by following classical amniocentesis rules, then 1 ml amniotic fluid per week of gestation was collected and sent to genetic laboratory for examination.[5] Pregnant women who had Rh inconsistency were administered 300 microgram anti-D ampoule intramuscularly as a single dose. Fetal loss occurred within two weeks after the procedure was evaluated as the complication associated with the procedure.

The evaluation of the first trimester screening test (double test) was performed by checking PAPP-A and free beta human chorionic gonadotrophin (hCG) in maternal blood at 11-14 weeks of gestation and calculating risk together with fetal nuchal thickness and presence of nasal bone in ultrasonography. The crown-rump length was used to evaluate the week of gestation of the fetus. Invasive procedure for karyotype determination depending on the week of gestation was recommended to the pregnants who have risk higher than 1/270 for delivering baby with Down syndrome. Chorionic villus sampling was carried out primarily in pregnants who were appropriate for the sampling and had appropriate placental localization. Amniocentesis was applied to patients who did not had appropriate placental localization and referred to our hospital at advanced week of gestation.

The evaluation of the second trimester screening test (triple test) was performed by measuring the levels of alpha-fetoprotein (AFP), hCG and estriol in maternal blood at 15-20 weeks of gestation. Biparietal diameter was measured in order to determine the week of gestation of the fetus. Invasive procedure for karyotype determination depending on the week of gestation was recommended to the pregnants who have risk higher than 1/270 for delivering baby with Down syndrome. Amniocentesis was performed to the pregnants who were up to 20 weeks of gestation while cordocentesis was recommended to the pregnants who were at higher weeks of gestation.

Amniotic fluid was cultured for long-term by means of flask method by using at least two different culture media. Twenty metaphase areas (providing that at least from 2 different cultures) from those obtained after mitotic retention and harvesting processes at about 10th day were analyzed by using image analysis system in terms of numerical and structural chromosomal abnormalites. The results were reported in accordance with ISCN nomenclature (International System for human Cytogenetic Nomenclature).

Results

It was found that the median age of 83 cases who had amniocentesis was 30 (range: 18-42) years. Median weeks of gestation was 17 (range: 16-20). Among 83 amniocentesis invasive performed, tissue culture was successful in 80 cases. Success rate in culture during amniocentesis was found as 96.4%. In cases where reproduction was detected, there was chromosomal abnormality in 10% of them (8/80).

The biggest group in terms of the indication was consisted of cases who had high risk at second trimester screening test. Amniocentesis for karyotype determination was applied to 30 cases (36.1%) found to have high risk at second trimester screening test, and chromosomal abnormality was found in 2 cases (6.6%). This indication was followed by the increased risk at first trimester screening test (34.9%, 29/83). In these cases, the rate of chromosomal abnormality was 13.8% (4/29). Amniocentesis indications have been shown in the **Table 1**.

Karyotyping results showed that there was trisomy 21 in the amnions of 5 cases, trisomy 21 mosaicism (46 XY+47XY[+21] and 46 XX+47XX[+21]) in 2 cases, and Turner mosaicism (45 XO+46 XY) in one case. While 46XY karyotype was found in 31 amniotic materials and 46XX in 40 of them, 46XX inversion 9 was found in one sample. The results of karyotype analysis have been shown in the **Table 2**.

None of 83 cases had maternal and fetal complications after the amniocentesis procedure.

Table 1. Amniocentesis indications in tertiary care unit in Manisa in 2012.

Indications	Number	%
Increased risk of second trimester screening test	30	36.1
Increased risk of first trimester screening test	29	34.9
Detecting abnormality during ultrasonography	13	15.6
Family request	8	9.6
Increased risk of quadruple test	2	2.4
History of delivery with chromosomal abnormality	2	2.4
Total	**83**	**100**

Discussion

Amniocentesis is the most applicable, with the least complication and the oldest prenatal diagnosis procedure in practice. Amniocentesis for karyotyping purpose is generally carried out at 16-20 weeks of gestation. It has been showed that the fetal loss rate associated with the invasive with amniocentesis applied during this period caused 1% more risk compared to the group which did not have amniocentesis.[3] There is about 0.1% amnionitis leak and 1-2% amniotic leak after amniocentesis. However, amniotic leak spontaneously stops usually between 48 and 72 hours by bed rest. Persistent amniotic fluid leak rarely may cause ascending infection and oligohydramniosis.[6] In such case, patient is followed up closely after the procedure and the hydration is increased. It was seen in cases with vaginal bleeding or amniotic leak that abortion rate may increase up to 18% and fetal loss rate up to 40%.[7] No fetal loss or maternal complication was seen in our procedures for a total of 83 cases within a year without regarding invasive to placenta.

In terms of all our amniocentesis cases, culture result could be obtained from 80 out of 83 cases. Culture success rate in amniocentesis was found as 96.4%. This success rate is consistent with the results of Güven et al. which was 98% and of Cengizoğlu et al. which was 99.[8,9] The reason for low success rate was found to be the laboratory errors. These patients were informed about the situation and cordocentesis was recommended due to

Table 2. Karyotype analysis results in tertiary care unit in Manisa in 2012.

Karyotype analysis result	Number	%
46XX	40	50
46XY	31	38.8
Trisomy 21	5	6.3
Trisomy 21 mosaicism	2	2.5
Turner mosaicism	1	1.2
Inversion 9 (46XX)	1	1.2
Total	**80**	**100**

advanced weeks of gestation. Current 3 patients accepted cordocentesis and normal karyotype results were obtained.

When all amniocentesis karyotype results were evaluated, we found chromosomal abnormality in 10% of our cases. This result was generally higher than other studies. Yayla et al. found chromosomal abnormality as 3.68% while Güven et al. found this rate as 3.2%.[8,10] The reason for this high rate in chromosomal abnormality is considered as the increased risk at first trimester screening test, because the chromosomal abnormality rate was 6.6% in the amniocentesis performed due to increased risk at second trimester screening test while it was 13.8% at first trimester screening test.

In our study, high rate of second trimester screening test (triple test) was found as the most frequent amniocentesis indication. This test is performed based on some biochemical markers which are in maternal serum at 15-20 weeks of gestation and secreted by mother-fetus unit, and its sensitivity is 60% with 5% error margin for Down syndrome.[11] This sensitivity rate is quite low compared to first trimester screening test (double test). Although the double test is considered as the most useful screening test in the world, it is the second amniocentesis indication at our clinic. It should be aimed to make this screening test with higher sensitivity widespread in further years by training and practice programs for obstetricians working in our region. On the other hand, although chorionic villus sampling is the primary diagnostic method after first trimester screening test in practice, pregnant women may refer to such reference centers as our hospital for various reasons after 14 weeks of gestation. Therefore, the increased risk at first trimester screening test has been detected as the highest rate at our center as amniocentesis indication. In addition, advanced maternal age which was seen as an amniocentesis indication with a rate of about 50-60% previously.[12-14] has been requested as an amniocentesis indication by 10% of families which is not considered as an amniocentesis indication. When the reason was asked to them, they stated that they wanted this procedure since all of them were above 35 years old. Since our hospital was a tertiary reference center, these patients are referred to our hospital due to the indication of advanced maternal age. These patients who have anxiety are informed by us. Even though detailed information is provided, all patients requested amniocentesis. None of them had chromosomal abnormality found in amniocentesis. Detailed information should certainly be given and patients should be redirected to screening test no matter what old they are.[15]

Finally, one case whose screening test results were normal was found to have chromosomal abnormality in amniocentesis since the markers for Down syndrome were positive (increased nuchal edema = 7.1 mm) in the ultrasonography performed on 19 weeks of gestation. This shows the significance of detailed ultrasonography performed at advanced weeks of gestation. Therefore, prenatal diagnosis should be established according to the week of gestation for all pregnants who are found to have abnormalities in ultrasonography such as increased nuchal edema, ventricular septal defect, cystic hygroma, ventriculomegaly, hydrops fetalis, and duodenal atresia etc.

Conclusion

Consequently, the sensitivity of first trimester screening test is high, and pregnants should have the test at 11-14 weeks of gestation. During these weeks, the first option in positive screening test should be chorionic villus sampling. Second option is to perform amniocentesis by waiting until 16 weeks of gestation in case of the lack of experience, non-availability of a reference hospital and difficulty to reach placental localization. If there is no sufficient experience and reference center for first trimester screening test, or if the patient is seen for the first time after 14 weeks of gestation, quadruple screening test should be performed to the patient.[16] Since quadruple screening test is not common as second trimester screening test (triple test), triple test is used in our country and our region currently during these weeks of gestation. Especially the patients who were found to have low risk by second trimester screening test (triple test) should undergo detailed ultrasonography at following weeks and chromosomal abnormality markers should be checked.

Conflicts of Interest: No conflicts declared.

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