Retrospective Analysis of 2295 Cases with Invasive Prenatal Diagnosis

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Abstract

Objective: Retrospective evaluation of the results of the chorion villus sampling, amniocentesis, and cordocentesis of 2295 cases performed for prenatal diagnosis.

Methods: Between 2001 and 2007 (first 6 months) 54 cases of genetic chorion villus sampling, 2086 cases of genetic amniocentesis and 155 cases of cordocentesis were evaluated according to indications, success of karyotyping and the results of the karyotyping.

Results: The majority of indication was high risk in triple screening test (n= 835, %36), abnormal ultrasonographic examination (n=493, %21), and advanced maternal age (n=490, %21) in all pregnant, respectively. High risk in triple screening test was the major indication in the cases that amniocentesis performed, abnormal ultrasonographic examination in the cases that cordocentesis and chorion villus sampling were performed. Tissues cultures were not successful in 64 of 2086 cases evaluated by AS, 10 of 155 cases evaluated by KS, 5 of 54 cases evaluated by CVS. Cultures were successful 2226 of 2305 cases (%96.4). Chromosome aberration were detected in 98 of 2216 cases (%4.4). 52 (%2.3) of this chromosomal aberration were number abnormalities, 46 of were structural abnormalities. The most frequent chromosomal abnormality was trisomy 21 in the number abnormalities and pericentric inversion of chromosome 9 in structural abnormalities. Karyotype aberration rate was higher in abnormal ultrasonographic examination (%8.8), advanced maternal age- high risk in triple screening test (%5.1) and advanced maternal age (%3.1). Chromosomal abnormality rate was %2.6 in the most common prenatal diagnosis indication (high risk in triple screening test).

Conclusion: The majority of indication was high risk in triple screening test (%36), abnormal ultrasonographic examination (%21), and advanced maternal age (%21) in all pregnant, respectively. Tissues cultures were successful in %96.4 of cases. Chromosome aberration were detected in %4.4 of cases.

Keywords: Chorion villus sampling, amniocentesis, cordocentesis, and chromosome aberration.

İnvazif prenatal tanı yöntemleri uygulanan 2295 olgunun retrospektif analizi

Amaç: Prenatal tanı amacıyla koryon villus örneklemesi (CVS), amniyosentez (AS), kordosentez (KS) uygulanan 2295 olgunun retrospektif analizi.

Yöntem: 2001–2007 (ilk 6 ay) tarihleri arasında kromozom analizi amacıyla CVS yapılan 54, AS yapılan 2086 ve KS yapılan 155 gebenin endikasyon, karyotipleme başarısı ve karyotip sonuçlarının retrospektif olarak değerlendirilmesi.

Bulgular: Çalışmamızda üçlü testte yüksek risk (n=835, %36), anormal ultrasonografik bulgu (n=493, %21) ve ileri anne yaşı (n=490, %21), prenatal tanı yapılan tüm gebeler için en sık görülen endikasyonlardır. Amniyosentez yapılan vakalarda en sık endikasyon üçlü testte yüksek risk (n=816) iken KS ve CVS yapılan vakalarda anormal ultrasonografik bulgu (n=110, n=45) ilk sırayı aldı. Amniyosentez yapılan 2086 olgunun 64 (%3)'üne, kordosentez yapılan 155 olgunun 10 (%6.4)'una, CVS yapılan 54 olgunun 5 (%9.2)'ine

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olmak üzere prenatal tanı amacı ile gönderilen 2295 hastanın 79 (%3.4)'una sonuç verilememiştir. Tüm olgularda elde ettiğimiz kültür başarısı %96.6'dır. Prenatal tanı için sitogenetik çalışma yapılan ve sonuç verilen 2216 olgunun 98 (%4.4) inde kromozom anomalisi saptanmıştır. Bu kromozom anomalilerinin 52 (%2.3) tanesi sayısal anomali iken, 46 (%2.1) tanesi yapısal anomalidir. Sayısal anomaliler içinde en sık görülen karyotip Trizomi 21 iken yapısal anomaliler içinde kromozom 9'un perisentrik inversiyonudur. Endikasyonlara göre en sık kromozom anomalisi saptanan ilk üç grup sırasıyla anormal ultrasonografik bulgu (%8.8), ileri anne yaşı-ÜTYR (%5.1) ve ileri anne yaşı (%3.1)'dır. En sık prenatal tanı endikasyonunu oluşturan grupta (üçlü testte yüksek risk) kromozomal anomali görülme oranı %2.6 olarak tespit edildi.

Sonuç: Çalışmamızda üçlü testte yüksek risk (%36), anormal ultrasonografik bulgu (%21) ve ileri anne yaşı (%21), prenatal tanı yapılan tüm gebeler için en sık görülen endikasyonlardır. Tüm olgularda elde ettiğimiz kültür başarısı %96.6'dır. Prenatal tanı için sitogenetik çalışma yapılan ve sonuç verilen gebelerin %4.4'ünde kromozom anomalisi saptanmıştır.

Anahtar Sözcükler: Koryon villus örneklemesi, amniyosentez, kordosentez, kromozom anomalisi.

Introduction

The primary aim in prenatal diagnosis is to diagnose as early as possible and to make the necessary decision according to the result. The important thing is not to regard the methods applied as a tool to end the pregnancy, but to obtain the right information about the fetus and help the family to make their own decision in accordance with the personal, social and ethical principles.[1] Prenatal diagnosis methods are divided into two parts called invasive and non-invasive methods. The most important ones of the non-invasive methods are ultrasound studies and biochemical tests done on the blood of mother. With the multi-centered studies including many European countries the effectiveness of USG in prenatal diagnosing was examined and it was shown that 50% of USG findings and fetal syndromes can be diagnosed without using other methods.[2,3] Nowadays, in the second trimester (14-22 weeks), Triple test consisting of AFP, total HCG and unconjugate Estriol level values is a commonly used prenatal scanning test.[4] In addition, quadruple test formed by adding inhibin-A to these parameters and in the first trimester, nuchal test in which PAPP-A (pregnancy associated placental protein-A), B-hCG free beta hCG and nuchal thickness are evaluated together are done. That scanning tests are non-invasive and economic has reduced the necessity of using invasive methods.

It has become possible to obtain knowledge about fetal karyotype through the invasive methods used in prenatal diagnosis. In the first and second trimesters, in order to prenatal diagnosis, Chorion Villus Sampling (CVS), Amniocentesis (AS) and Cordocentesis (CS) have been applied as the invasive classical methods performed these days. Each method is different in terms of time of feasibility, convenience of feasibility, period of getting laboratory results and complications. Amniocentesis is an invasive method which is done between the 16-20th weeks and often used in prenatal diagnosis. Ager and Oliver have stated in their intermediate evaluations that the risk of fetal loss has increased by 0.2-2.1% in the amniocentesis group in comparison with the control group.[5,6] Chorion Villus Sampling (CVS) has been preferred because it can be performed early (at about the 8th week of pregnancy), there is no direct intervention in fetus and so no hurt, and so much material can be obtained, which is regarded as an advantage for the DNA studies. In the CVS material, both cells at the metaphase or other stages can be directly evaluated and cytogenetic studies can be done following culture examinations.[7,8] Smidt-Jensen et al. found the fetal loss risk as 2.5% at transcervical approach, 2.3% at transabdominal approach and they determined that the difference between them was meaningless.[9] CS or cord blood sampling (from 21st week on) is an indis-

pensable method for prenatal diagnosis studies. In the cases of being late for applying for the prenatal diagnosis and being unsuccessful with AS, CS comes into effect. Although it is known that in problematic pregnancies, the fetal mortality depending on invasive procedure may be higher, it is accepted that common average is 1-2%.[10,11]

In this study, the results of the cytogenetic analysis done with the aim of prenatal diagnosis in the Department of Medical Genetics in Erciyes University Medical School between 2001-2007 (first six months) have been evaluated retrospectively.

Methods

Between the years of 2001 – 2007 (first six months), in the Department of Gynecology and Obstetrics of Gevher Nesibe Research Hospital and other hospitals the records of 2295 pregnant women from whom the samples were taken after doing chorion villus sampling, amniocentesis and cordocentesis with the aim of prenatal diagnosis, and whose samples were given a chromosome analysis were retrospectively studied in terms of the success of cell culture, invasive indications and their genetic results.

All the pregnant women and their husbands were informed of the procedure and possible complications before the application, and a written consent was taken from the couples who had accepted the application. All the pregnant women were examined in terms of being a hepatitis porter and having an Rh disagreement. A detailed genetic sonogram was done. The chorion villus sampling was performed with the transabdominal chorion villus sampling method technique and about 10 mg of fetal tissue was taken into the transport medium.[12] The amniocentesis was done in accord with the classical amniocentesis rules on the 16th–20th weeks. In order to reduce the maternal contamination, the first 2ml was aspired into a separate injector. Then a total of 18–20 ml of amniotic liquid was taken in to two different injectors. Cordocentesis was performed by taking 2 cc of fetal blood into the injector which has 0.5 cc heparin, depending on the localization of placenta, either from the free cord or from the spot 1–2 cm away from the place where the cord enters the placenta between the 19th – 28th weeks of pregnancy.[12] At the end of all these applications, the unsensitised pregnant women who have Rh incompatibility were given 300 microgram of anti-D immunoglobulin G.

The samples taken for the cytogenetic studies were cultivated in proper methods and harvested. For the evaluation of the numeral and structural disorder of the chromosomes in all the cases, at least 20 metaphase plates were examined with the computerized analysis system.

Results

The indications and average ages at which the invasive procedures were settled and pregnancy weeks of the pregnant women whose prenatal diagnostic applications had been made were shown in the table 1. High risk in triple test (n=835), abnormal ultrasonographic examination (n=493) and advanced maternal age (n=490) are the leading indications in the triple test in all the prenatal diagnostic applications. While in the cases to whom amniocentesis was applied, the most frequent indication is the high risk in triple test (n=816), in the cases to whom CS and CVS were applied the abnormal ultrasonographic examination (n=816) took the first place.

The result couldn't be given to 79 out of 2295 cases (3.4%) who were sent with the aim

Table 1. Indications, average age and pregnancy weeks of the pregnants that performed prenatal diagnosis.

Indication of prenatal diagnosis	CVS	AS	KS	Total	Percent
Triple Test Risk		816	19	835	36
Abnormal USG	45	338	110	493	21
Maternal age risk		481	9	490	21
Maternal age risk-Triple test risk		314	5	319	14
Down syndrome in the family history	3	61	3	67	3
Dysmorphic child in the family history	4	41	3	48	2
Child with muscular disorders in the family history		12		12	0,5
Repeated pregnancy loss		7		7	0,3
Others *	2	16	6	24	1
TOTAL	54 (%2)	2086 (%91)	155 (%7)	2295	
Mean mother age	27.84	31.17	27.15		
Mean pregnancy week	12;57	18.95	25.61		

**Others; IUGR, double test risk, mother anxiety, child with chromosomal abnormality in the family history, toxoplasmosis, drug using in the pregnancy, intrauterine transfusion, Rh incompatibility*

Table 2. Chromosomal abnormalities in all pregnants.

Caryotype	CVS	AS	CS	Total
47,XY,+21 veya 47,XX,+21	4	24	3	31
46,XX,inv9(p11;q12) or 46,XY,inv9(p11;q12)		13		13
47,XY,+18 or 47,XX,+18		4	3	7
46,XY,16qh+ or 46,XX,16qh+	1	4	1	6
45,X		3		3
47,XXX		1	2	3
45,XY,der(13;14)(q10;q10)		3		3
46,XY,15ps+ or 46,XX,15ps+		3		3
47,XY,+13 or 47,XX,+13	1	1		2
69,XXX		1	1	2
46,XX,14ps+		2		2
46,XX,21ps+ or 46,XY,21ps+		2		2
46,XY,22ps+ or 46,XY,22ps+		2		2
46,XX[95]/47,XX,+18[5]			1	1
47,XY,+mar		1		1
47,XXY		1		1
46,XY[84]/47,XYY[16]		1		1
46,XX,der(14;21)(q10;q10),+21		1		1
46,XX,der(17)t(10;17)(q24.2;p13)mat		1		1
46,XX,der(9)t(7;9)(p15.3;p24)mat,16qh+			1	1
46,XX,t(1;3)(q23;21)		1		1
46,XX,t(1;16)(p13.3;p13)			1	1
46,XY,t(4;9)(pter;q34)		1		1
46,XX,t(12;22)(p11.2;p12)		1		1
46,XY,t(16;17)(q13;q23)		1		1
46,XY,t(7;15)(q11,2;q26.3)		1		1
46,XX[60]/46,XY[40]		1		1
46,XY[80]/46,XX[20]		1		1
46,XX,inv9(p11;q12),15ps+		1		1
46,XY,9qh+		1		1
Total	6	77	13	96

of prenatal diagnosis: These were 64 out of 2086 cases to whom amniocentesis was applied (3%), 10 out of 155 cases to whom cordocentesis were applied (6.4%), 5 out of 54 cases to whom CVS was applied (9.2%). The culture success we obtained from these cases was 96.6%.

Chromosome anomaly was determined in 98 out of 2216 cases (4.4%) on whom cytogenetic studies were done for prenatal diagnosis and to whom the results were given. While 52 of these chromosome anomalies (2.3%) were numerical anomalies, 46 (2.1) were structural anomalies (Table 2). While the most commonly karyotype seen among numerical anomalies is trisomy 21, the one among structural anomalies is the pericentric inversion of chromosome 9. The three groups in which the most common chromosome anomaly was determined according to the indications were abnormal ultrasonographic examination (8.8%), advanced maternal age-triple test risk (5.1%) and advanced maternal age respectively. In the group which formed the most common prenatal diagnosis indication (the high risk in the triple tests), the rate at which chromosome anomaly can be seen was determined as 2.6%. According to the indications of the pregnant women who were given prenatal diagnosis, the frequency at which chromosome anomaly can be detected was shown in Table 3.

Discussion

In our work, the high risk in the triple test (36%), abnormal ultrasonographic examination (21%) and advanced maternal age (21%) are the most commonly seen indications for the pregnant mothers given a prenatal diagnosis. In the literature, there are varied rates in the studies where the amniocentesis indications have been evaluated. The first three most frequent indications in the work of Sener et al. are the same as the ones in our work.[13] While the first three indications in the work of Kose et al. are the advanced maternal age (42.3%), pathology in the second scanning test (28.3%) and pathologic ultrasound finding (8.6%) respectively, the first three indications in the work of Guven et al. are the triple test with a high risk, anomaly seen in the ultrasonogram and advanced maternal age.[14,15] When the frequency of cordocentesis indications in the literature were studied, Guven et al. showed the advanced age and Yayla et al. showed the abnormal ultrasonographic examination as the most frequent indication.[15,16] In our work, abnormal ultrasonographic examination has taken the first place.

Table 3. Chromosomal abnormality ratio according to their indication.

Indication of Prenatal Diagnosis	Number of pregnant women	Number of fetus with chromosomal abnormality	Percent of fetus with chromosomal abnormality (%)
Üçlü testte yüksek risk (ÜTYR)	805	21	2.6
Anormal ultrasonografik bulgu	475	42	8.8
İleri anne yaşı	471	15	3.1
İleri anne yaşı-ÜTYR	308	16	5.1
Down sendromlu çocuk doğurma öyküsü	67	1	1.5
Anomalili çocuk doğurma öyküsü	48	1	2
Kas hastalıklı çocuk doğurma öyküsü	11	0	0
Tekrarlayan gebelik kaybı	7	0	0
Diğerleri	24	2	8
Toplam	2216	98	4.4

When all the cases to which the prenatal diagnosis had been evaluated, 79 of 2295 patients couldn't be given a result. The culture success we obtained is 97% in AS, 93.6% in CS and 90.8% in CVS. That is totally 96.6%. It has been stated in the literature that the AS culture success of Cengizoglu et al. is 99%, the amniocentesis culture success of Guven et al. and Yuce et al. is 98% and the AS culture success of Yayla at al is 92.7%, their cordocentesis culture success is 85%.[17,15,18,16] Their cordocentesis and fetal karyotyping success is about 90%.[19] In the literature, the culture success in CVS samples of Türkyılmaz et al. is 88%. We think that the culture failure has been due to the contaminations of the amnion liquid during the material extraction, earlier bleeding, insufficient material extraction, contamination, sample keeping and problems during the transport conditions.

The chromosome anomaly rate seen in all our pregnant women who have been given prenatal procedures is 4.4%. The chromosome anomaly rate seen in AS cases in the Literature is between 2-5.8% (the chromosome anomaly rate in AS series of Yayla et al. is 3.6%, that of Basaran et al. is 3.5%, that of Guven et al. is 2%).[16,20,15] The chromosome anomaly rate seen in the cordocentesis cases is 8.2-15.25%.[21,15,16] Turkyılmaz et al. determined that the chromosome anomaly rate in the chorion villus sampling is 8%.

The frequency at which chromosome anomaly is seen in the pregnant women who have been given AS because of the abnormal ultrasonographic examination varies from 8.7% to 35.6%.[22,23,16,24] The 8.8% rate determined in our work seems to comply with the literature. And this also shows how important especially a detailed ultrasonogram scanning is. Karyotype anomaly was found in 2.6% cases of the patients who had been given amniocentesis and cordocentesis because of the triple test with a high risk. This rate varies between 1.5% and 10 in the literature.[13,14,16] It is thought that this wide range is due to the threshold value and the standardization difference between the laboratories. As Sener et al. stated, the importance of a triple test must be questioned by the other centers. While the frequency at which the chromosome anomaly is seen in the pregnant women who have been given a chromosome analysis owing to the indications of the triple test with a high risk is 2.6%, this frequency has become 5.1% at the advanced maternal age – triple test risk. We think that this is because the frequency at which down syndrome appears together with advanced age has increased.

The reason in 51-60% of the recurrent abortions is the chromosome anomaly.[25,26] In our work, the 7 pregnant women who had recurrent abortions were directly given AS, and the karyotypes of these 7 women were found to be normal.

Conclusion

The majority of indication was high risk in triple screening test (%36), abnormal ultrasonographic examination (%21), and advanced maternal age (%21) in all pregnant, respectively. Tissues cultures were successful in %96.4 of cases. Chromosome aberration were detected in %4.4 of cases.

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