Second Trimester Genetic Amniocentesis: Five- Year Experience of a Maternal- Fetal Medicine Unit

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OBJECTİVE: The objective of this study is to identify the annual variations amniocentesis indications such as change in maternal age, cytogenetic results, and other indications. Another outcome is the investigation of the relationship between indications for amniocentesis and the distribution of chromosomal abnormalities.

STUDY DESIGN: This study was designed as a retrospective analysis of amniocentesis results of the 1667 pregnant patients between January 2007 and December 2012 in the Süleymaniye Women's Health Education and Research Hospital. The karyotype results, indications for intervention and complications during procedure were reported.

RESULTS: Total chromosome abnormalities were detected in 101 cases out of 1667 patients which correspond to a 6.1% of the total results. Distribution of the chromosomal abnormality detection rate with respect to the amniocentesis indication was 4.2%; in the abnormal first trimester screening tests group; 5.3% in the abnormal second trimester maternal serum screening group, 18.7% in the fetal malformations in previous pregnancy group, and 7.1%. in the abnormal ultrasound findings group.

CONCLUSION: Amniocentesis is the most common invasive procedure for prenatal diagnosis. Although the advanced maternal age is still an important indication, there has been significant development of both new markers and technology making this indication for amniocentesis questionable. Prenatal ultrasonography for the soft markers of chromosomal aneuploidy in association with the maternal serum biochemical screening tests should be evaluated during the decision process for amniocentesis.

Key Words: Genetic amniocentesis, Chromosomal abnormalities, Detection rate

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Introduction

Prenatal cytogenetic diagnostic methods for the diagnosis of fetal chromosomal anomalies have been used reliably over the last 40 years. Amniocentesis is an invasive procedure that requires removing a sample of amniotic fluid to obtain fetal cells for chromosome analysis.

Schatz introduced amniocentesis for the treatment of polyhydramniosis in 1882,[1] and he also used it for the diagnosis and treatment of Rhesus incompatibility. Fuchs and Riis reported the usage of amniocentesis in sex determination in 1956.[2] Chromosomal analysis of human tissues using a cell culture obtained by amniocentesis was reported by Steele and Bregs in 1966. Since 1967, amniocentesis has been used as a prenatal diagnosis method.[3,4] Amniocentesis is currently the most commonly used invasive prenatal diagnostic procedure. It is performed between the 15th and 20th gestational weeks. It was shown that fetal cells obtained by amniocentesis could be cultured and grown; therefore it can be used for the diagnosis of chromosomal anomalies as well as the detection of carriers for genetic disorders via DNA and enzyme analysis. In the past, advanced maternal age and adverse obstetric history were the two leading indications for amniocentesis. Nowadays, the importance of amniocentesis has been highlighted due to the advances in maternal screening with the using of serum markers and ultrasonography. With an increased awareness of anomalous children affected by environmental pollution, and postponement of the pregnancy age, patients are becoming conscious and ask more for prenatal diagnosis. Although amniocentesis is considered to be a relatively safe procedure, fetal loss and maternal complications can

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rarely occur after the operation.[5] Although there has been an ongoing development in the ultrasonography technology and there are advances in obtaining fetal DNA from maternal blood, the invasive prenatal tests are still important and sometimes crucial for genetic counseling.

The objective of this study was to identify the changes in the indications for amniocentesis over a 5 year period. Another outcome was the investigation of the relationship between indications for amniocentesis and the distribution of reported chromosomal abnormalities.

Material and Method

A retrospective cohort study was designed for the evaluation of 1667 amniocentesis procedures between January 2007 to December 2012 in the maternal- fetal unit of Süleymaniye Women's Health Education and Research Hospital. Medical records were reviewed with a focus on indications, karyotype results and complications.

As a standard protocol in our unit, information was given to all patients and their partners about application of technique, fetal loss rate, other risks and complications of the procedure. Informed consent was taken from all of the patients. All of the patients were evaluated for rhesus group before the procedure and 300 mcg anti D immunoglobulin was given in the case of Rh incompatibility.

In every case during the mentioned time period, a standard protocol of our unit was applied. A Voluson 730 Expert ultrasound device was used during the amniocentesis procedure. The fetal biometric measurements, fetal cardiac activity and location of the placenta were evaluated before the procedure. A spinal needle of 22- Gauge was used for the amniocentesis. The abdomen area was sterilized twice with the octenidine dihydrochloride solution and the ultrasound probe was covered with a sterile surgical glove. Local anesthesia was not used during the intervention. A 20 cc syringe was used to aspirate the amniotic fluid following removal of the needle stylet. The first 2 cc of fluid was discharged and then another syringe was used to aspirate 15 to 20 cc of amniotic fluid. During the procedure attention was paid not to puncture a fetal part or fetal cord in a fluid pouch. If a fetal part or cord was identified, we used the transplacental route. The obtained materials were examined in a genetics center using the long term cell culture, GTL and CBG banding methods. An ultrasound examination was performed after every amniocentesis to exclude possible complications and to confirm the presence fetal cardiac activity. One week after the procedure this examination was repeated.

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) Software version 16.5 (SPSS Inc., USA) for PC.

Results

Were analyzed 1667 amniocentesis performed between January 2007 and December 2012. Total chromosome abnormalities were detected in 101 cases (6.1%) with 6.1% overall positive rate of abnormal cytogenetic findings (101/1667). The annual distribution of the amniocentesis cases are shown year by year in table 1.

The maternal age of the patients varied from 17 to 51 years old, with the average age being 33.9 (±6.3) years old. The gestational age of the fetuses varied from 16.1 to 24.2 weeks, with an average being 18.2 (±2.4) weeks.

Complications that occurred in 15 days after the procedure were spontaneous abortion in three patients, after four weeks in five patients. Total fetal loss ratio that occurred one to four weeks after the procedure was 0.5%.

The number of cases and detection rate of chromosomal abnormalities in different indications for amniocentesis are shown in table 2. Abnormal ultrasound findings were found in 683 patients (40.9%), 49 patients had an abnormal karyotype with a detection rate of 7.1%. Abnormal second trimester biochemical markers were detected in 594 patients (35.6%) and 32 of them (5.3%) had an abnormal karyotype. Abnormal first trimester biochemical markers were identified in 304 patients (18.2%) and 13 of them (4.2%) had chromosomal abnormalities. The advanced maternal age group (age ≥ 35 year old) was 3.8% (65/1667) of the total study population and in 3% of this group a chromosomal abnormality was detected (Table 2).

Amniocentesis was performed by transamniotic entry in 1506 (90.3%) cases and transplacental entry was used in 161 (9.7%) cases. The procedure was performed successfully in

Table 1: The annual distribution of total cases of amniocentesis

Year	2007 (%)	2008 (%)	2009 (%)	2010 (%)	2011 (%)	2012 (%)	Total (%)
Number of cases	127	441	340	253	255	251	1667
	7.6%	26.4%	20.3%	151%	15.2%	15%	100%

%: Proportion

the first attempt in 1615 (96.8%) cases and it was accomplished in 52 (3.2%) cases in the second attempt.

The prevalence of chromosomal abnormalities in different indications for amniocentesis were as follows: Abnormal first trimester screening test 4.2% (13/304); abnormal second trimester biochemical markers 5.3% (32/594); previous child with anomaly 18.7% (3/16); and abnormal ultrasound findings 7.1% (49/683) (Table 3).

Among the cases with chromosomal abnormalities, 53 (52.4%) were numerical abnormalities and 48 (47.6%) were structural abnormalities. Among the numerical abnormalities: 35 cases (34.6%) were with trisomy 21; 7 cases (6.9%) were identified as trisomy 18; 2 cases (1.9%) with trisomy 13; 6 cases (5.9%) with 47 XXY and 3 cases (2.9%) with 45 X (Turner syndrome) were diagnosed out of 53. For the structural abnormalities: 9 cases (8.9%) of reciprocal translocations; 1 case (0.9%) of robertsonian translocations; 16 cases (15.8%) of inversion; 6 cases (5.9%) of deletion; 7 cases (6.9%) of duplication and 8 cases (7.9%) of marker chromosome were diagnosed out of 48 (Table 3). Trisomy 21 prevalence with respect to amniocentesis indication was as follows in descending order, 54.2% (19/35) in abnormal ultrasound findings; 34.2% (12/35) in abnormal second trimester biochemical markers; 11.4% (4/35) in abnormal first trimester screening test. Trisomy 18 was found commonly in cases with the indications of abnormal ultrasound findings 71.4% (5/7); abnormal first trimester screening test 28.5% (2/7); abnormal second trimester biochemical markers 14.2% (1/7). The abnormality of 47 XXY, 45 X, inversions and duplications were frequently noted in cases with the indication of abnormal ultrasound findings (Table 4).

Table 2: Number of cases and detection rate of chromosomal abnormalities in different indications for amniocentesis

Indication	Case number	Proportion (%)	Abnormal karyotype	Detection rate (%)
AUF	683	41	49	7.1
ASTBM	594	35.7	32	5.3
AFTST	304	18.2	13	4.2
AMA	65	3.9	2	3.1
PCA	16	0.9	3	18.7
FHCA	6	0.3	-	-
Total	1667	100	101	6.1

AUF: Abnormal ultrasound findings, ASTBM: Abnormal second trimester biochemical markers, AFTST: Abnormal first trimester screening test, AMA: Advanced maternal age, PCA: Previous child with anomaly, FHCA: Family history of chromosomal abnormalities. Abnormal biochemical markers in maternal serum in the second trimester = increased risk maternal triple- marker Down' s screening test (≥ 1/270); advanced maternal age (≥ 35 years old).

Table 3: Distribution of numerical and structural chromosomal abnormalities.

	n	%
Numerical abnormalities	53	52.4
Trisomy 21	35	34.6
Trisomy 18	7	6.9
Trisomy 13	2	1.9
47, XXY	6	5.9
45, X	3	2.9
Structural abnormalities	48	47.6
Reciprocal translocation	9	8.9
Robertsonian translocation	1	0.9
Inversion	16	15.8
Deletion	6	5.9
Duplication	7	6.9
Marker chromosome	8	7.9
Total	101	100

n: Case number, %: Proportion.

Table 4: The detection rates of chromosomal abnormalities according to different indications.

Anomaly	AUF n/dr (%)	ASTBM n/ dr (%)	AFTST n/ dr (%)	AMA n/ dr (%)	PCA n/ dr (%)	FHCA n/ dr (%)
Trisomy 21	19(2.7%)	12(2.0%)	4(1.3%)	0	0	0
Trisomy 18	5(0.7%)	1(0.1%)	2(0.6%)	0	0	0
Trisomy 13	1(0.1%)	1(0.1%)	0	0	0	0
47, XXY	4(0.5%)	2(0.3%)	0	0	0	0
45, X	2(0.2%)	1(0.1%)	0	0	0	0
Reciprocal translocation	2(0.2%)	3(0.5%)	4(1.3%)	0	0	0
Robertsonian translocation	0	0	1(0.3%)	0	0	0
Inversion	10(1.4%)	5(0.8%)	0	0	1(6.2%)	0
Duplication	2(0.2%)	4(0.6%)	1(0.3%)	0	0	0
Marker chromosome	7(1%)	1(0.1%)	0	0	0	0

AUF: Abnormal ultrasound findings, ASTBM: Abnormal second trimester biochemical markers, AFTST: Abnormal first trimester screening test, AMA: Advanced maternal age, PCA: Previous child with anomaly, FHCA: Family history of chromosomal abnormalities. Abnormal biochemical markers in maternal serum in the second trimester = increased risk maternal triple- marker Down' s screening test (≥ 1/ 270); advanced maternal age (≥ 35 years old). n: Case number, dr: Detection rate.

Discussion

Amniocentesis which has been applied since 1800's, is the most frequent prenatal invasive test.[6] After the publication of the article titled 'role of amniocentesis in intrauterine detection of genetic disorders' by Naddler in 1970[7] genetic amniocentesis became routinely used in obstetrics. Although several efforts have been made for the development of ultrasound technology and the advancement in serum biochemical markers, we are still dependent on invasive procedures in prenatal diagnosis. With the widespread use of rapid genetic methods like polymerase chain reaction (PCR) and fluorescence in situ hybridization (FISH), invasive procedures like amniocentesis are becoming prevalent in prenatal diagnosis. Advanced maternal age, abnormal ultrasound findings, abnormal second trimester biochemical markers in maternal serum, abnormal first trimester screening tests, previous birth of an abnormal child and family history of chromosomal abnormalities are classical indications for amniocentesis. In our study, the most common indications for amniocentesis were found as abnormal ultrasound markers (40.9%) and abnormal second trimester biochemical markers in maternal serum (35.6%), followed by other indications such as; abnormal first trimester screening tests, advanced maternal age and previous child with anomaly. In literature, different indication ratios for amniocentesis were reported. In previous years the advanced maternal age was the most common indication for amniocentesis. Tongsong, also reported the advanced maternal age as the most common indication for amniocentesis (86.3%)[8] Contrary to their study, advanced maternal age was the fourth common indication for amniocentesis in our study. Hsieh et al.[10] highlighted the abnormal ultrasound findings as the most common indication for amniocentesis in their study. Their result was also consistent with our study.

Detection rates for chromosomal abnormalities in different indications for amniocentesis are given in Table 5 and our results were comparable with the literature.[9-14]

Ultrasound markers might be one of the best indicators for planning amniocentesis, since our study showed a high positive rate of abnormality when the indication for amniocentesis was chosen according to the abnormal ultrasound findings (7.1%). Our detection rate for this group was consistent with the study of Yang et al. (6.5%),[19] and higher than those mentioned by Karaoguz et al. (5.3%),[14] but lower than Tseng et al.'s study (8.9%)[11] and even lower than Hsieh et al.'s report (20.3%).[10] The possible reason for the significantly high abnormal cytogenetical findings in the abnormal ultrasound group of Hsieh et al.'s study might be caused by selection bias (Hsieh was a pioneer in obstetric ultrasound and high- risk pregnancy). The other reason may be due to the relatively limited number of cases in their study (n=2975) compared to Tseng et al's study (n=7028).[11] Progress in ultrasonographic examination has also resulted in an increased number of candidates for amniocentesis.[15] In this study; 40.9% amniocentesis was done due to abnormal findings depicted in ultrasound examinations. In the study of Yang, 7.9% chromosomal abnormalities were found when the indication for amniocentesis was chosen as abnormal ultrasound findings regardless of maternal age or maternal serum markers.[16] In this study; 7.1% chromosomal abnormalities were found in the abnormal ultrasound finding group.

Table 5: Amniocentesis of different studies, including the indications and their detection rates for chromosomal abnormalities.

	1987, Bell et al.[13]	1989, Kimet al.[12]	1992, Hsieh et al.[10]	2006, Tseng et al.[11]	2006, Karaoguz et al. [14]	Our study
n/ dr	1000(2.1%)	126(3.2%)	2975(3.0%)	7028(2.9%)	6041(3.0%)	1667(6.05%)
AUF/ dr	0	0	148(20.3%)	553(8.9%)	492(5.3%)	683(7.1%)
ASTBM/ dr	0	7	0	1500(2.6%)	2011	594(4.2%)
AFTST/ dr	0	0	0	0	0	304(4.2%)
AMA/ dr	750	74	1629(2%)	4026(2.31%)	3197	65(3.07%)
PCA/ dr	0	21	143(11.8%)	0	173	16(18.7%)
FHCA/ dr	100	0	0	0	0	6(0%)
ACVSR/ dr	0	2	0	0	0	0
IUFD/ dr	0	3	0	949(2.7%)	0	0
PAK/ dr	0	2	0	0	14	0
RME/ dr	0	0	153(5.3%)	0	0	0
Others	0	3	0	0	0	0

AUF: Abnormal ultrasound findings, ASTBM: Abnormal second trimester biochemical markers, AFTST: Abnormal first trimester screening test, AMA: Advanced maternal age, PCA: Previous child with anomaly, FHCA: Family history of chromosomal abnormalities, ACVSR: Abnormal CVS results, PAK: Parent with abnormal karyotype, RME: Radiation or medication exposure. Abnormal biochemical markers in maternal serum in the second trimester = increased risk maternal triple- marker Down' s screening test (≥ 1/ 270); advanced maternal age (≥ 35 years old); CVS = chorionic villus sampling. IUFD = intrauterine fetal death. %: percentage, n: Case number, dr: Detection rate

These results strongly advocate the need for amniocentesis in cases of abnormal ultrasound findings. In this study, we also confirmed the value of maternal serum screening for Down syndrome with a chromosomal anomaly detection rate of 5.3%, compared with 3.1% detection rate of advanced maternal age group.

Previous pregnancies affected by genetic abnormalities were also a good indicator for the decision of amniocentesis. In our study we had a limited number of cases (16 cases), however we found that 18.7% chromosomal anomaly in the previous pregnancies affected by genetic anomalies group.

In the study of Chang et al., where the indication for amniocentesis was family history of chromosomal abnormalities the detection rate was found to be 11.5%.[17] However we couldn't identify any chromosomal abnormality in patients when amniocentesis was done with the indication of family history of chromosomal abnormalities.

In the Van Dyke et al.'s study, the advanced maternal age group had the highest detection rate for numerical abnormalities (18), but in our study the abnormal ultrasound findings group had the highest detection rate for both the numerical and structural abnormalities.

In our analysis, cases with the indications based on abnormal ultrasound findings and abnormal second trimester maternal serum biochemical markers had a higher prevalence of trisomy 21, trisomy 18, trisomy 13, 47 XXY and 45 X, which was the same as the previous study.[17]

The small population size and the retrospective design of our study were the main limitations. In our study, among all indications the detection rate was the highest in abnormal ultrasound findings group. This result can be attributed to the population of patients referred to our hospital for amniocentesis. Most of the cases that were referred to our hospital for prenatal examination were evaluated in other centers and had abnormal ultrasound findings.

Fetal karyotype is an important diagnostic tool that should be offered to patients after genetic counseling and abnormal screening tests. The advanced maternal age was the primary and only indication for amniocentesis in the past. However, due to the development of maternal serum markers and identification of early fetal ultrasound findings with the sensitive ultrasonographic technology, the indications for amniocentesis have been shifting. If we look at recent statistics, the most common indication for amniocentesis is now stated as abnormal maternal serum markers. Also there is an increase for the contribution of abnormal findings that are detected by ultrasound as an amniocentesis indication.

In conclusion this study showed the importance of abnormal ultrasound findings and abnormal serum markers for the detection of chromosomal abnormalities. These findings are important because in the past maternal age was the only indicator for amniocentesis but now, abnormal ultrasound findings and serum markers have been more common as amniocentesis indications with the development of technology. As technologies advance, it is even more important to look at the abnormal ultrasound and serum markers as oppose to maternal age for amniocentesis. This five year study at a maternal- fetal unit of a maternity ward in Istanbul might be useful in estimating positive amniocentesis results before genetic counseling.

İkinci Trimester Genetik Amniyosentezi: Bir Maternal - Fetal Tıp Ünitesinin Beş Yıllık Deneyimi

AMAÇ: Bu çalışmanın amacı; amniyosentez endikasyonlarının yıllara göre değişimini ve farklı endikasyonlarla yapılan genetik amniyosentezlerin kromozom anomalisi yakalama oranının belirlenmesidir.

GEREÇ VE YÖNTEM: Çalışma retrospektif olarak dizayn edilmiş olup, Ocak 2007- Aralık 2012 tarihleri arasInda Süleymaniye Doğumevi Eğitim ve Araştırma Hastanesi'nde yapılan 1667 ikinci trimester genetik amniyosentezleri incelenmiştir. Genetik amniyosentez endikasyonları ve karyotip sonuçları kayıt altına alınmıştır.

BULGULAR: Belirtilen tarihler arasındaki 1667 ikinci trimester genetik amniyosentezinin 101'inde (%6,1) kromozom anomalisi tespit edildi. Farklı amniyosentez endikasyonları incelendiğinde kromozom anomalisi yakalama oranları şu şekilde bulundu; anormal ultrason bulguları olanlarda %7,1, anormal birinci trimester tarama testi sonucu olanlarda %4,2, anormal ikinci trimester maternal serum belirteçleri olanlarda %5,3 ve önceki gebeliğinde fetal malformasyon öyküsü olanlarda %18,7.

SONUÇ: Genetik amniyosentez invaziv prenatal tanıda en çok uygulanan prosedürdür. Geçmiş yıllarda en önemli amniyosentez endikasyonları arasında sayılan ileri anne yaşı, günümüzde yerini farklı endikasyonlara bırakmıştır. Minör belirteçlerin gelişen prenatal ultrasonografi ile belirlenebilmesi ile birlikte maternal serum belirteçlerinin kombinasyonu sonrasında amniyosentez endikasyonu koyulması uygun olacaktır.

Anahtar Kelimeler: Genetik amniyosentez, Amniyosentez endikasyonları, Kromozom anormallikleri

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