Evaluation of clinical and cytogenetic findings on 1,068 second-trimester amniocenteses in Southeast Turkey

M. Balkan[1], H. Akbas[1], S. Kalkanli[1], M.N. Sakar[2], M. Fidanboy[1], M.N. Alp[1], T. Budak[1]

[1]Department of Medical Biology and Genetics, Medical Faculty, [2]Department of Gynecology and Obstetrics Medical Faculty, University of Dicle, Diyarbakir (Turkey)

Summary

Objective: To investigate the indications of amniocentesis for the detection of chromosomal abnormalities among a sample of patients in Southeast Turkey. *Material and Methods:* Between 2004 and 2007, 1,068 second-trimester amniocentesis tests were performed in the Medical Biology and Genetics Department Laboratory at Dicle University. Amniotic fluids were cultured by using long-term tissue culture for prenatal diagnosis with cytogenetic analysis. The clinical and cytogenetic findings on 1,068 second-trimester amniocenteses were analyzed. The indications, the proportions of karyotypes according to indications and complications were summarized. *Results:* Among the 1,068 amniocentesis cases, the maternal age between 35 and 39 years was the most common age group (34.5%). Of the clinical indications abnormal maternal serum screening results were the most common indication for amniocentesis (37.6%). Of 52 cases (4.9%) with detected chromosomal aberrations, 39 were numeric (27 trisomies, 10 sex chromosome aberrations and two triploidies) and 13 were structural (2 reciprocal translocations, 2 Robertsonian translocations and 6 inversions). The highest detection rate of chromosome aberrations was in cases undergoing amniocentesis for abnormal maternal serum screening combined with abnormal ultrasound (US) findings (8.0%). *Conclusion:* This study suggests that complementary measures, such as routine antenatal US and maternal serum screening, should be added to increase the efficiency of genetic amniocentesis. Therefore, the study could be used for the establishment of a database for genetic counseling.

Key words: Amniocentesis; Chromosome aberrations; Genetic counseling; Prenatal diagnosis.

Introduction

Prenatal diagnosis with cytogenetic analysis, such as chorionic villus sampling, amniocentesis and cordocentesis has been recognized for more than 20 years as a safe and reliable method for couples at increased risk of giving birth to a child with a clinically significant chromosomal abnormality [1-4]. Of these methods, amniocentesis for chromosomal abnormalities remains the most common invasive prenatal diagnostic procedure today [2-16]. Accurate risk estimates for chromosomal abnormalities are important tools for the physician or obstetrician, who would need to make referrals to a prenatal genetic center [9]. The discovery of an abnormality allows the option of termination or, later in the pregnancy, more suitable obstetric management [8].

The most common indications for prenatal diagnosis with cytogenetic analysis include advanced maternal age (AMA), abnormal biochemical markers in the maternal serum, previous chromosomal abnormality and prenatal structural rearrangements [5-8, 10-15]. This study investigated the indications for amniocentesis for the detection of chromosomal abnormalities among a sample of patients in Southeast Turkey. Between 2004 and 2007, 1,068 amniocentesis tests were performed in the Medical Biology and Genetic Department Laboratory of Dicle University.

Revised manuscript accepted for publication May 31, 2011

Material and Methods

Study setting and population

The cytogenetic findings from 1,068 second-trimester amniocentesis cases obtained between 2004 and 2007 were reviewed. Amniocenteses were performed in various medical sites, but the majority (90%) were carried out at the Department of Gynecology and Obstetrics, Dicle University Hospital in the city of Diyarbakir, Southeast Anatolia Region of Turkey.

All samples were analyzed for cytogenetic analyses in the Medical Biology and Genetics Department Laboratory at Dicle University. The laboratory provides a prenatal service to obstetrics-gynecology departments of different hospitals in Diyarbakır and its surrounding provinces in Southeast Turkey. The laboratory appraisal of the amniotic fluid was the responsibility of the Department of Human Genetics. The findings in these cases are summarized in the Results section.

A detailed interview was conducted with all couples before amniocentesis, and a detailed medical history was obtained. The complications and risks of amniocentesis were explained to the family in detail. The risk of abortion due to the procedure was also explained to the families. They were informed that, if an abnormality was identified, a legal termination of pregnancy could be offered. Informed consent for genetic testing was obtained from all patients.

The main indications for amniocentesis with cytogenetic analysis in this study included; AMA, that is, if the mother was ≥ 35 years old at the expected date of confinement; abnormal screening markers (α-fetoprotein, human chorionic gonadotropin, and/or unsaturated estriol) in maternal serum; abnormal ultrasound (US) findings; previous fetus/child with chromosomal aberrations; previous abnormal and/or mentally retarded child, previous neonatal death or stillbirth; maternal anxiety.

Cultures and harvesting and cytogenetic analysis

The amniotic fluids were cultured by using long term tissue culture in three different flasks containing 2.5-3 ml of medium Bioamf (Biological Industries, Israel) as the basal medium supplemented with Bioamf supplement (Biological Industries), 1% 200 mm L-glutamine (Gibco, New York, USA), 100 U/ml penicillin (Biological Industries), and 100 µg/ml streptomycin (Biological Industries), using the technique described earlier [17]. Cultures were harvested when colonies were sufficient (at least 15 colonies), 9-14 days after seeding. Chromosomes were prepared in the usual manner. Routine diagnosis was based on examination of GTG-banded chromosome from at least 20 cultured metaphase cells from a minimum of two independent culture dishes. In some cases, chromosomes analyses were performed by using both the GTG-banding and Ag-NOR chromosome-banding techniques. In the cases of mosaic karyotypes, 30-100 metaphase spreads were analyzed. The karyotypes were described according to the International System for Chromosome Nomenclature [18].

All chromosomal abnormalities detected by karyotype analysis have been classified into numerical and structural abnormalities. Chromosomal variants that are not clinically significant, such as enlarged heterochromatin on various chromosomes and enlarged satellites, were not included. Frequencies of observed chromosomal abnormalities were then calculated for each indication and considered second trimester risk estimates.

Genetic counseling

Genetic counseling was provided by obstetricians to all couples before the amniocenteses were performed in the Department of Gynecology and Obstetrics. Those patients that were identified as having pregnancies with chromosomal anomalies received post-amniocentesis genetic counseling in the Department of Medical Biology and Genetics. Our prenatal genetic counseling center was established in 1994 to serve patients [19].

The couples in this study were informed of the nature of the study, and the signatures of the couples for their informed consent were obtained. All of the patients interviewed clearly understood that the research was independent of their care, and that their participation in the study would not affect their care in any way. All protocols were IRB approved. None of the patients declined to participate.

Results

As previously stated, 1,068 second-trimester amniocentesis cases were analyzed at the cytogenetics lab in our prenatal genetic center between 2004 and 2007. After receiving genetic counseling, cases were selected to undergo a prenatal cytogenetic study. The majority of the women in our study had completed formal education. Of the 1,068 women; 398 (37.3%) had graduated from a university; 249 (23.3%) from a high school; 210 (19.7%) from a primary school, and 211 (19.8%) had no formal education.

Of the 1,068 women who had amniocentesis, 34.5% (n = 368) had a maternal age between 35 and 39 years, which was the most common age group, followed by age 30-34 (23.6%, 252), 25-29 (20.2%, 216), older than 40 (13,3%, 142), 20-24 (6.5%, 70), and 19 years or younger (1.9%, 20) (Table 1).

The gestational ages at the time when the amniocentesis was performed were: ≤ 15 weeks in 5% of cases, 16 weeks in 28%, 17 weeks in 33%, 18 weeks in 14%, 19 weeks in 7%, 20 weeks in 4%, 21 weeks in 2%, 22 weeks in 3%, 23 weeks in 2% and ≥ 24 weeks in 2%.

The indications for the amniocenteses are shown in Table 2. Among these, the most common clinical indication for amniocentesis was abnormal maternal serum screening results (37.6%), followed by AMA (24.9%), abnormal maternal serum screening combined with AMA (14.0%), abnormal US findings (7.3%), maternal anxiety

Table 1. — *Age distribution.*

Maternal age (years)	Amniocenteses (n)	%
≤ 19	20	1.9
20-24	70	6.5
25-29	216	20.2
30-34	252	23.6
35-39	368	34.5
≥ 40	142	13.3
Total	1068	100.0

Table 2. — *Number of amniocenteses cytogenetically analyzed according to indication.*

Indication	Amniocenteses (n)	Proportion (%)
Advanced maternal age (AMA) (≥ 35)	266	24.9
Abnormal maternal serum screenings results	402	37.6
Abnormal ultrasonographic (US) findings	78	7.3
Previous fetus/child with chromosomal aberrations	34	3.2
Previous abnormal and/or mentally retarded child	32	3.0
Previous neonatal death or stillbirth	21	2.0
Maternal anxiety	35	3.3
Abnormal maternal serum screening + AMA	149	14.0
Abnormal maternal serum screening + Abnormal US findings*	25	2.3
AMA + Abnormal US findings	26	2.4
Total	1068	100.0

*US anomaly identified at the time of pre-amnio US?

Table 3. — *Types and frequencies of chromosomal abnormalities.*

Karyotypes	Number (n)	%
Numerical abnormalities	39	75
Autosomal abnormalities	27	51.9
Trisomy 21	20	38.5
Trisomy 18	5	9.6
Trisomy 13	2	3.8
Triploidy	2	3.8
Sex chromosome abnormalities	10	19.2
Turner syndrome	5	9.6
Classic	4	7.7
Mosaic[a]	1	1.9
Klinefelter syndrome	3	5.8
Triple X syndrome	2	3.8
Structural rearrangements	13	25
Reciprocal translocation	2	3.8
Robertsonian translocation[b]	2	3.8
Inversion	6	11.5
Deletion	2	3.8
Supernumerary marker chromosome	1	1.9
Total	52	100.0

[a]45,X/46,XX;

[b]One of the Robertsonian translocations was unbalanced: 46,XX,+13,der(13;14)(q10;q10).

Table 4. — *Types and frequencies of chromosome abnormalities according to the indications.*

Indications	Total number (n)	Chromosomal abnormalities n (%)	Type of abnormalities (n)
AMA (≥ 35)	266	19 (7.1%)	Trisomy 21 (11) Trisomy 18 (2) Trisomy 13 (1) Turner (2) Klinefelter (1) Triple X Syndrome (1) 46,XY,inv(9)(p13q13) (1)*
Abnormal maternal serum screenings results	402	12 (3.0%)	Trisomy 21 (6) Klinefelter (2) Turner (2) 46,XY,inv(9)(p11q11) (1)** 46,XY,inv(9)(p11q13) (1)*
Abnormal US findings	78	6 (7.7%)	Trisomy 18 (1) Trisomy 21(1) Trisomy 13 (1) Triploidy (1) 46,XY,del(18)(p?) (1)* Triple X Syndrome (1)
Previous fetus/child with chromosomal aberrations	34	2(5.9%)	46,XY, t(3;7)(q24;q36) (1)a 46,XY,inv(9)(p11q13) (1)*
Previous abnormal and/or mentally retarded child	32	1 (3.2%)	46,XY,inv(9)(p11q13) (1)*
Previous neonatal death or stillbirth	21	1 (4.8%)	45,XY,der(13;14)(q10;q10) (1)b
Maternal anxiety	35	1 (2.9%)	46,XY,inv(9)(p11q11) (1)**
Abnormal maternal serum screening + AMA	149	6 (4.0%)	Trisomy 21 (1) Turner (1) 46,XY, t(3;18)(p23;p11) (1)c 46,XY,del(18)(p?) (1) (1)* Trisomy 18 (2)
Abnormal maternal serum screening + Abnormal US findings	25	2 (8.0%)	Triploidy (1) 46,XX,+13,der(13;14)(q10;q10) (1)d
AMA + Abnormal US findings	26	2 (7.7%)	Trisomy 21 (1) 47,__,+mar (22) (1)*
Total	1068	52	

[a] de novo reciprocal translocation, [b] de novo Robertsonian translocation, [c] familial reciprocal translocation in association with a balanced paternal reciprocal translocation, [d] familial Robertsonian translocation in association with a balanced maternal reciprocal translocation, *de novo cases, **familial cases.

(3.3%), previous fetus/child with chromosomal aberrations (3.2%), previous abnormal and/or mentally retarded child (3.0%), AMA combined with abnormal US findings (2.4%), abnormal maternal serum screening combined with abnormal US findings (2.3%), and previous neonatal death or stillbirth (2.0%).

In 1,068 of our cases, except six, cells were able to be grown in culture (99.4%). The frequencies by classification for chromosomal abnormalities are shown in Table 3. Of the 1,068 amniocenteses, 1,016 cases (95.1%) showed normal diploidy and 52 cases (4.9%) showed chromosomal abnormalities. Among these chromosomal abnormalities, numerical and structural abnormalities were seen in 39 and 13 cases, respectively. The majority of chromosomal abnormalities were autosomal trisomies (51.9%, 27/52). Trisomy 21 syndrome was the most common abnormality (38.5%, 20/52). Edward syndrome and Patau syndrome were found in five and two cases, respectively. Triploidy syndrome was found in two cases (69, XXY and 69, XXX). In cases with sex chromosomal abnormalities (10 cases), five cases had Turner syndrome (4 classic and 1 mosaic), three Klinefelter syndrome (classic), and two triple X syndrome. Total structural rearrangements were found in 13 cases (25.0%, 13/52). Among structural chromosomal rearrangements, reciprocal translocations were detected in two cases and Robertsonian translocations in two cases. One of the Robertsonian translocations was unbalanced (trisomy 13). Marker chromosome was found in one case, deletions in two cases and inversions were present in six cases.

Table 4 gives the frequencies of chromosome abnormalities according to the indications. Of 25 cases with abnormal maternal serum screening combined with abnormal US findings, two cases resulted in chromosomal abnormalities, which showed the highest positive predictive value (8.0%) among indications, followed by abnormal US findings (7.7%), AMA combined with abnormal US findings (7.7%), AMA (7.1%), previous fetus/child with chromosomal aberrations (5.9%), previous neonatal death or stillbirth (4.8%), abnormal maternal serum screening combined with AMA (4.0%), previous abnormal and/or mentally retarded child (3.2%), abnormal maternal serum screenings results (3%), and maternal anxiety (2.9%).

Discussion

Prenatal diagnosis has become a major aid in genetic counseling, and thus several important areas of technology have evolved, such as cytogenetic prenatal diagnosis, by using analysis of cultured cells from the amniotic fluid at mid-trimester. Because of its high reliability and safety record with the lowest fetal loss and embryonic damage, amniocentesis has become the most common practice for prenatal diagnosis [13, 19]. This technique was established in 1989 in Turkey and in 1994 in our department.

The reports on prenatal diagnosis of amniocentesis have revealed that the incidence of chromosomal abnormalities ranges between 1.0% and 6.7% [5, 6, 8-16]. In this study, it was found that 4.9% of 1,068 cases had chromosomal abnormalities, which was similar to the data of Kromberg *et al.* [16] (4.9%) and Acar *et al.* [2] (5.2%). The wide variation in incidence of chromosome abnormalities may have accounted for changes in cytogenetic technology, sensitivity of US, advent and utilization of maternal serum screening, gestational age at diagnosis, etc.

Prenatal diagnosis by chromosomal analysis has been increasingly used in obstetric practices for the diagnosis and treatment between 15 and 18 gestational weeks since it became available using amniocentesis in 1967 [5]. In the 1980s, amniocentesis was used primarily for those in advanced maternal age groups, at least 35 years old [5]. So far, other recent reports have still shown that prenatal diagnosis of chromosomal disorders has been performed mainly for pregnancies at an AMA [8-10, 13, 20]. In the present study it was determined that abnormal maternal serum screening was the most common indication for amniocentesis, followed by AMA (Table 2). This finding was similar to the results of previous studies [5, 13, 21-23]. Maternal serum marker screening has been accepted as the prominent indication for amniocentesis among obstetricians over time [5, 9]. In particular, this test has made remarkable progress both as a routine prenatal screening program and a detection technique in our center. The cost of abnormal maternal serum screening was paid by the patient herself if she did not have health insurance, or covered by private insurance, or funded by a state-supported agency in Turkey.

It has earlier been reported that abnormal US findings showed the highest detection rate for chromosomal abnormalities in prenatal diagnosis, at ranges between 5.3% and 8.9% [5, 8, 9, 14]. In the present study, abnormal maternal serum screening combined with abnormal US findings, AMA combined with abnormal US findings and abnormal US findings showed the highest positive predictive values among the clinical indications, at 8%, 7.7%, and 7.6% respectively (Table 4). Today, highly sensitive US technology can detect many fetal anomalies, which eventually necessitate amniocentesis.

In cases with maternal anxiety, there should be a proper diagnosis in consideration of psychiatric stress which could affect the family [5]. This study found 35 women (3.3%) who requested amniocentesis due to the indications above (Table 2).

Among numerical autosomal abnormalities, Trisomy 21 syndrome was the most common abnormality found in our study (Table 3). This result is similar to some previous results reported in the literature [5, 6, 8, 9, 13, 15]. In addition, in the present study it was found that the prevalence of Trisomy 21 cases (1.9%) among all the study population slowly increased from the results of previous studies [5, 9, 13-15, 24], which might be due to a remarkable progress in the maternal serum screening test and US techniques.

Turner syndrome was the most common of the sex chromosomal abnormalities in our study (Table 3). Although the phenotype varies from normal female to full manifestations of Turner syndrome, it has been reported that the abnormal phenotypic rate of prenatally diagnosed cases is about 14% at birth [5]. Some authors have concluded that the dynamics surrounding sex aneuploidies with a low risk of an abnormal clinical phenotype probably differ from those surrounding Down syndrome and aneuploidies in which a severe clinical phenotype is expected [25]. In addition, it has been demonstrated in long-term follow-up studies that the postnatal development of cases with sex chromosome aneuploidies is mostly normal [25, 26]. These types of empirical evidence are likely to have influenced genetic counseling strategies.

Translocations were present in four fetuses, including two Robertsonian translocations and two reciprocal translocations (Table 3). One of the Robertsonian translocations was balanced and de novo: 45,XY,der(13;14)(q10;q10 and the other case was unbalanced and familial: 46,XX,+13,der(13;14)(q10;q10) in association with a balanced maternal Robertsonian translocation. Of the two reciprocal translocations, one case was de novo: 46,XY, t(3;7)(q24;q36) and the other case was familial translocation: 46,XY, t(3;18)(p23;p11) in association with a balanced paternal reciprocal translocation (Table 4). The problem is entirely different in parents carrying a translocation [15]. As is well known, there is information about the de novo translocations as in the carrier whose risk of having an aneuploid live birth lies in the medium range, 5%-10% and in the highest range, 35%. This risk is, of course, very variable as it depends on the breakpoints of each translocation. Some translocations are indeed lethal for the embryo when unbalanced and will never be observed, while others are not as damaging [15]. Families in our study were informed about these risks.

Inversion 9 was identified in 11.5% (6/52) (Table 3). These karyotypes were generally considered normal variants without phenotypic effects on the individuals carrying these aberrations [27]. Nevertheless, there have been also debates on the association between these karyotypes and various clinical problems. The prevalence of inversion chromosome 9 in a normal population was reported as 1.65%, and higher incidence in aborted fetuses with normal karyotype (3.31%) and couples with a history of more than two spontaneous first trimester abortions (3.19%) [28]. In another study, there were patients having inv(9) accompanied by delayed development or mental

retardation (25.0%), congenital anomaly (23.1%), giving birth to babies with an inv(9) (15.4%) and habitual abortion (7.7%) [27]. However it was not possible to confirm whether inv(9) was responsible for these clinical findings.

In summary, prenatal diagnosis by amniocentesis in the past was performed mainly for AMA. However, due to the development of maternal serum markers and sensitive US technology, the indications for amniocentesis are changing. For daily practice, our data corroborates the importance of prenatal diagnosis in light of the well defined indication categories, offering amniocentesis to mothers in order to evaluate prognosis and to give accurate information concerning the child to be born and the risk for further pregnancies.

References

[1] Ndumbe F.M., Navti O., Chilaka V.N., Konje J.C.: "Prenatal diagnosis in the first trimester of pregnancy". *Obstet. Gynecol. Surv.*, 2008, *63*, 317.

[2] Acar A., Balci O., Gezginci K., Onder C., Capar M., Zamani A. *et al.*: "Evaluation of the results of cordocentesis". *Taiwan J. Obstet. Gynecol.*, 2007, *46*, 405.

[3] Savacı S., Yüksel Ş., Yeşilada E., Kaygusuz E., Gülbay G.: "Prenatal diagnosis evaluation at medical biology and genetic Department Laboratory of Inönü University for two years". *Inönü Üniversitesi Tıp Fakültesi Dergisi*, 2008, *15*, 19.

[4] Yıldırım G., Güngördük K., Gül A., Aslan H., Ceylan Y., Gedikbaşı A.: "Results of cordocentesis at our department: evaluation of 260 cases". *J. Turk. Ger. Gynecol. Assoc.*, 2008, *9*, 224.

[5] Han S.H., An J.W., Jeong G.Y., Yoon H.R., Lee A., Yang Y.H. *et al.*: "Clinical and cytogenetic findings on 31,615 mid-trimester amniocenteses". *Korean J. Lab. Med.*, 2008, *28*, 378.

[6] Daniilidis A., Karydas H., Zournatzi V., Tantanasis T., Giannoulis C., Tzafettas J.: "A four-year retrospective study of amniocentesis: one centre". *Hippokratia*, 2008, *12*, 113.

[7] Odabası A.R., Yüksel H., Demircan S., Temocin K., Bal F., Yapıcı S. *et al.*: "The results of second trimester genetic amniocentesis procedure: adnan menderes University Experience with the results of 22 Centers in Turkey". *Turkiye Klinikleri J. Gynecol. Obstet.*, 2007, *17*, 196.

[8] Tseng J.J., Chou M.M., Lo F.C., Lai H.Y., Chen M.H., Ho E.S.: "Detection of chromosome aberrations in the second trimester using genetic amniocentesis: experience during 1995-2004". *Taiwan J. Obstet. Gynecol.*, 2006, *45*, 39.

[9] Karaoguz M.Y., Bal F., Yakut T., Ercelen N.O., Ergun M.A., Gokcen A.B. *et al.*: "Cytogenetic results of amniocentesis materials: incidence of abnormal karyotypes in the Turkish collaborative study". *Genet. Couns.*, 2006, *17*, 219.

[10] Turhan N.O., Eren U., Seckin N.C.: "Second-trimester genetic amniocentesis: 5-year experience". *Arch. Gynecol. Obstet.*, 2005, *271*, 19.

[11] Gunduz C., Cogulu O., Cankaya T., Bora E., Karaca E., Alpman A. *et al.*: "Trends in cytogenetic prenatal diagnosis in a reference hospital in Izmir/Turkey: a comparative study for four years". *Genet. Couns.*, 2004, *15*, 53.

[12] Park I.Y., Shin J.C., Kim S.C., Ahn H.Y., Moon H.B., Park C.H. *et al.*: "Cytogenetic analysis in 3,503 cases of midtrimester amniocentesis: CUMC experience (II)". *Korean J. Obstet. Gynecol.*, 2004, *47*, 96.

[13] Park S.Y., Kim J.W., Kim Y.M., Lee M.H., Lee B.Y. *et al.*: "Frequencies of fetal chromosomal abnormalities at prenatal diagnosis: 10 years experiences in a single institution". *J. Korean Med. Sci.*, 2001, *16*, 290.

[14] Yang Y.H., Ju K.S., Kim S.B., Cho Y.H., Lee J.H., Lee S.H. *et al.*: "The Korean collaborative study on 11,000 prenatal genetic amniocentesis". *Yonsei Med. J.*, 1999, *40*, 460.

[15] Caron L., Tihy F., Dallaire L.: "Frequencies of chromosomal abnormalities at amniocentesis: over 20 years of cytogenetic analyses in one laboratory". *Am. J. Med. Gene.*, 1999, *82*, 149.

[16] Kromberg J.G., Bernstein R., Jacobson M.J., Rosendorff J., Jenkins T.: "A decade of mid-trimester amniocentesis in Johannesburg. Prenatal diagnosis, problems and counselling". *S. Afr. Med. J.*, 1989, *76*, 344.

[17] Verma R.S., Babu A.T.: "Issue culture techniques and chromosome preparation". In: Verma R.S., and Babu A. (eds.). Human Chromosomes Principles and Techniques. 2nd edition, New York: McGraw-Hill, 1995, 6.

[18] ISCN 2005. An International System for Human Cytogenetic Nomenclature (Cytogenetic & Genome Research). Shaffer L.G., Tomnerup N., Basel, Karger, 2005.

[19] Balkan M., Akbas H., Isi H., Oral D., Turkyilmaz A., Kalkanli S. *et al.*: "Cytogenetic analysis of 4216 patients referred for suspected chromosomal abnormalities in Southeast Turkey". *Genet. Mol. Res.*, 2010, *11*, 1094.

[20] Kessler R.G., Sanseverino M.T.V., Leistner-Segal S., Magalhães J.A.A., Giugliani R.: "Prenatal diagnosis of fetal chromosomal abnormalities: Report of an 18-year experience in a Brazilian public hospital". *Gen. Mol. Biol.*, 2008, *31*, 829.

[21] Marthin T., Liedgren S., Hammar M.: "Transplacental needle passage and other risk factors associated with second trimester amniocentesis". *Acta Obstet. Gynecol. Scand.*, 1997, *76*, 728.

[22] Tongsong T., Wanapirak C., Sirivatanapa P.: "Amniocentesis related fetal loss; a cohort study". *Obstet. Gynecol.*, 1998, *92*, 64.

[23] Sener K.T., Durak B., Tanir H.M., Tepeli E., Kaya M., Artan S.: "Results of amniocentesis in 7 years period in Eskisehir Osmangazi University". *Perinatol.*, 2006, *14*, 170.

[24] Marical H., Douet-Guilbert N., Bages K., Collet M., Le Bris M.J., Morel F. *et al.*: "Second-trimester prenatal screening for trisomy 21 using biochemical markers: a 7-year experience in one cytogenetic laboratory". *Prenat. Diagn.*, 2006, *26*, 308.

[25] Quadrelli R., Quadrelli A., Mechoso B., Laufer M., Jaumandreu C., Vaglio A.: "Parental decisions to abort or continue a pregnancy following prenatal diagnosis of chromosomal abnormalities in a setting where termination of pregnancy is not legally available". *Prenatal. Diagnosis*, 2007, *27*, 228.

[26] Brun J., Gangbo F., Wen Z.Q., Galant K., Taine L., Maugey-Laulom B.: "Prenatal diagnosis and management of sex chromosome aneuploidy: a report on 98 cases". *Prenatal. Diagnosis*, 2004, *24*, 213.

[27] Kim S.S., Jung S.C., Kim H.J., Moon H.R., Lee J.S.: "Chromosome abnormalities in a referred population for suspected chromosomal aberrations: a report of 4117 cases". *J. Korean Med. Sci.*, 1999, *14*, 373.

[28] Yamada K.: "Population studies of INV(9) chromosomes in 4,300 Japanese: incidence, sex difference and clinical significance". *Jpn. J. Hum. Genet.*, 1992, *37*, 293.

Address reprint requests to:
M. BALKAN, Ph.D.
Department of Medical Biology
and Genetics,
Medical Faculty University of Dicle
21280, Diyarbakır (Turkey)
e-mail: balkanmah@gmail.com
mahbal@dicle.edu.tr