Experimental
&
Clinical Article

Importance of Routine Ultrasonography in Detecting Fetal Karyotype Abnormalities in Low Risk Pregnancies

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OBJECTIVE: The present study aims to evaluate the importance of USG findings, in estimating cytogenetic abnormality risks in pregnancies otherwise carrying low risk for aneuploidy.

STUDY DESIGN: We reviewed a number of most commonly observed soft markers and structural abnormalities in pregnant women with low risk who underwent invasive prenatal diagnostic tests for USG abnormality in the period from January 2002 to December 2008, retrospectively. In 179 of all cases (9.95%), cytogenetic analysis was recommended because of USG abnormality.

RESULTS: 76 patients had structural abnormalities, 95 had soft markers and 8 had intra-uterine growth restriction. We detected 12 aneuploidies in fetuses with structural abnormalities and 1 aneuploidy in fetuses with soft markers.

CONCLUSION: We concluded that, although the presence or absence of soft markers can substantially modify the risk of fetal aneuploidy, one or more structural abnormalities inevitably have high risk for aneuplodies as independent factor for low-risk pregnancies.

Key Words: Aneuploidy, Fetal ultrasonography, Prenatal diagnosis

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Introduction

Chromosomal abnormalities have a significant association with perinatal mortality and infant handicap. Therefore, various methods have been used to identify women at risk of carrying a fetus with chromosome abnormality, including consideration of maternal age, first and second trimester maternal serum screening tests and prenatal ultrasound.[1,2] When prenatal karyotyping indication was restricted to advanced maternal age (35 years old or older), only approximately 20% of all abnormal fetuses could be identified.[3] In addition, any couple can have an affected pregnancy and most babies with Down syndrome are born to women under the age of 35. Therefore, the first and second trimester maternal serum screening tests were used as a means to identify pregnancies at increased risk for certain birth defects and chromosomal abnormalities. Thus an additional 30-40% of fetuses with chromosome abnormality became identifiable.[4,5] Prospective studies indicate that fetal nuchal translucency measurement can be an effective screening test for trisomy 21 with detection rates of 80% for a 5% false positive rate.[6,7]

Although the fetal ultrasound for genetic disorders has not been standardized, recent reports have suggested that the sensitivity for detecting trisomy 21 ranges were between 60% and 93%.8 Trisomy 21 (Down syndrome) is the most common karyotypic abnormality in live-born infants (1 per 800 live births) and other sonographically detectable aneuploidies include trisomy 13, trisomy 18, monosomy X, and triploidy.[2,8]

First- (11-14 weeks) and second-trimester (18-23 weeks) USG screening findings of fetal chromosome abnormalities include structural abnormalities and/or soft markers of fetal aneuploidy.[9,10] Soft markers of fetal aneuploidy may be seen in normal fetuses, these are also often transient and non specific.[1,9] Therefore risk assessment depending on the presence of a single marker is difficult. Because USG markers are also common among karyotypically normal fetuses, it may not be clear when genetic diagnosis should be offered. Prenatal ultrasonography (USG) can also detect many fetal malformations, about 90% of which occur in fetuses born to parents with no recognizable risk factors. With improvements in sonographic resolution and improver operator skill, a greater range and number of fetal anomalies are now being detected.[7]

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In this study, we aimed to evaluate the importance of USG findings in estimating the risk of cytogenetic abnormalities, in pregnancies otherwise carrying low risk for aneuploidy.

Material and Method

We reviewed a number of most commonly accepted soft markers and structural abnormalities among the pregnant women who underwent invasive prenatal diagnostic tests due to sole USG findings in the period from January 2002 to December 2008, retrospectively. During this period, 1798 case applications for prenatal genetic diagnosis and 1688 amniotic fluid (AF), 68 fetal blood (FB) and 42 chorionic villus (CV) samples were obtained for cytogenetic analysis in our institution. In this study we included 179 couples who have no known genetic risk and abnormal maternal screening results, thus accepted as low risk pregnancies, but presence of soft marker or structural abnormality was detected by ultrasound. In each case invasive diagnostic test was performed either chorionic villus sampling (CVS), amniocentesis (AS) or fetal blood sampling (FBS). Two groups were created; one was soft marker, other was structural abnormalities. Moderate and severe intra-uterine growth restriction (IUGR) with fetal biometry <10th centile has been reported as soft marker or separate USG marker in previous studies,[1,11] We preferred to evaluate this finding separately.

First trimester screening for aneuploidy was performed between 11 and 14 weeks and second trimester screening was performed between 18 and 23 weeks of gestation. Cytogenetic analysis was performed by routine direct and/or short and/or long term culture methods; depending on gestational age at CVS, AS or FBS. Chromosomes were obtained from at least two different cultures from each sample and analyzed after GTG banding. Karyotype was established after numerical analysis of 30 methaphases and structural analysis of 5 methaphases. We classified the fetal USG abnormalities in two groups as soft markers and structural abnormalities (Table 1). The patients had undergone formal genetic counseling before the invasive procedure and post diagnostic counseling after the karyotype results. Written informed consent form was obtained from each couple during this period. In the presence of isolated fetal soft markers when there was actually no indication for an invasive diagnostic test (such as the presence of isolated choroid plexus cyst), prenatal genetic diagnosis was performed because of family request.

Table 1: The classification of fetal ultrasonographic findings which were the most frequently observed in our study.

Organ System	Structral Abnormalities	Soft Markers
CNS	Ventriculomegaly (≥15mm) Holoprosencephaly Microcephaly (biparietal diameter (BPD) <1st percentile and HC/FL < 2.5th percentile) Agenesis of corpus callosum Abnormal posterior fossa- dandy walker complex	Choroid plexus cyst Mild ventriculomegaly
Musculoskeletal	Hand and feet anomalies– syndactyly, clinodactyly, clenched fist, radial ray aplasia, clubfoot and rocker-bottom foot	Short long bones
Face	Cleft palate and lips, micrognathia, macroglossia, hypo- and hypertelorism, low set ears, small ear	Absent nasal bone
Neck	Cystic hygroma	Nuchal fold- thickening
Cardiac	Endocardial cushion defect, atrioventricular septal defect, ventricular septal defect, hypoplastic left heart syndrome, tetralogy of Fallot, and other complex cardiac anomalies	Echogenic intracardiac focus
Gastrointestinal tract	Esophageal and duodenal atresia, small bowel obstruction, diaphragmatic hernia and omphalocele	Echogenic bowel
Genitourinary tract	Moderate to severe hydronephrosis, dysplastic renal disease, and renal agenesis	Mild pyelectasis
Others	Hydrops	Single umbilical artery

Results

Prenatal genetic diagnosis was performed on total 179 cases out of 1798 (9.95%), because of abnormalities detected at obstetric USG without any genetic risk factors. 159 amniotic fluid samples, 14 fetal blood samples and 6 chorionic villus samples were studied for chromosome analysis. Fetal loss due to invasive procedures was not observed. In all cases resulting in a fetus with chromosomal aneuploiy the parents opted for termination of pregnancy.

76 (42.45%) cases had fetal structural abnormalities, 95 (53.07%) had fetal soft markers and 8 (4.46%) had IUGR. We detected 12 (14.28%) aneuploidies as chromosomal abnormalities in the fetuses with structural abnormalities and 1 (1.05%) aneuploidy in the fetuses with soft markers (Table 2). Number of patients, material types and number of samples with detected aneuploidy in each sample group are shown in Table 3. We did not detect structural chromosomal abnormalities, and there was one case (Case 2) with mosaic karyotype including a 45,X cell line (Table 2).

USG findings according to which our common indications for prenatal genetic diagnosis were classified are listed in table 1. The most common fetal structural abnormality observed was in the cardiovascular system at the second trimester screening periods. Omphalocele as a common finding was observed in seven cases which were detected with other abnormalities except one. Five of these cases had abnormal karyotype but two had normal karyotype (Table 2).

There were 56 cases with isolated soft marker including increased nuchal fold thickness (20/38), choroid plexus cysts (11/22), mild ventriculomegaly (7/14), echogenic bowel (6/11), echogenic intracardiac focus (5/8), short femur (2/10), single umbilical artery (2/10) oligohidramnios (2/4), polyhidramnios (1/6) at the second trimester screening.

IUGR was observed in 8 fetuses (7 AF cases and 1 FB case). Among the 7 fetuses, three had isolated IUGR; rest four had other associated fetal structural abnormalities in AF cases. The mean maternal age of the cases was 26, 4 years (range, 23- 32 years) and at the time of amniocentesis the mean gestational age was 20 weeks (range, 18^{+6} - 21^{+1}). All of the cases had normal karyotype. There was one case that had undergone cordocentesis at the 29th weeks due to IUGR and structural abnormalities. Cytogenetic analysis revealed normal karyotype in the fetus.

The mean maternal age of the cases in which amniotic fluid samples were obtained, was 26, 5 years (range, 18-34 years) and at the time of amniocentesis the mean gestational age was 19 weeks (range, 16^{+1} - 24^{+6}). In one case with twin pregnancy amniotic fluid was obtained at the 12th gestational week during selective fetocite (case 4). Among the cases who had undergone amniocentesis due to the presence of fetal soft markers (n: 84), 51 cases had one fetal soft marker and 33 cases had two or more fetal soft markers. The karyotype in one case with two fetal soft markers was demonstrated as 47,XX,+21 (Case 12), in the others, cytogenetic analysis revealed normal karyotype.

In amniocentesis cases with fetal structural abnormalities (n: 68), 30 fetuses had one abnormality, 28 had at least two structural abnormalities and 10 had one structural abnormality together with soft markers. The karyotype was abnormal in 11 cases including ten cases with autosomal aneuploidy and one case with monosomy X (Table 2 and 3).

Table 2: Abnormal fetal karyotype results and ultrasonographic indications for prenatal diagnosis

Case No	Indications	Age (Year)	Gestational age (week)	Material type	Karyotype
1	Cystic hygroma, unilateral pleural effusion	18	16	AF	45. X
2	Anencephaly, omphalocele, scoliosis	28	14	CV	45.X/46.XX
3	Omphalocele	34	16	AF	47.XY.+13
4	Omphalocele, micrognathia, megacystis, iNT, flat face	28	12	AF	47.XY.+13
5	Unilateral choroid plexus cysts, unilateral renal agenesis	24	24	AF	47.XX.+18
6	Choroid plexus cysts, micrognathia, VSD, rockerbottom foot	25	21	AF	47.XY.+18
7	Bilateral choroid plexus cysts, VSD	32	18	AF	47.XY.+18
8	Omphalocele, choroid plexus cysts, clench hand	20	19	AF	47.XX.+18
9	Omphalocele, single umbilical artery	24	22	AF	47.XY.+18
10	Renal pyelectasis, AVSD	27	24	AF	47.XX.+21
11	AVSD	31	23	AF	4.,XX.+21
12	Choroid plexus cysts, short femur	34	17	AF	47.XX.+21
13	Cystic hygroma	34	16	AF	47.XX.+21

AF: Amniotic fluid, AVSD: Atrioventricular septal defect, CV: Chorionic villus, iNT: increased nuchal translucency, VSD: Ventricular septal defect

Table 3: The mean maternal age and the number of cases with aneuploidy with respect to the sample material

Material	USG finding	Number of Cases	Number of Cases with Detected Aneuploidy (%)
AF	Structural Abnormality	68	11 (16.17)
	Soft Marker	84	1 (1.19)
	IUGR	7	-
FB	Structural Abnormality	7	-
	Soft Marker	6	-
	IUGR	1	-
CV	Structural Abnormality	1	1 (100)
	Soft Marker	5	-

AF: Amniotic fluid, CV: Chorionic Villus, FB: Fetal blood, IUGR: Intra-uterine growth restriction

Fetal aneuploidy was not detected in the cordocentesis cases (n: 13) with soft markers (n: 6) and structural abnormalities (n: 7). The mean maternal age of the cases was 27.5 years (range, 19- 34 years) and, at the time of cordocentesis, the mean gestational age was 24 weeks (range, 18^{+0}- 32^{+0}). CNS abnormalities (Ventriculomegaly, 5 cases; agenesis of corpus callosum, 2 cases) and extremity abnormalities (Rocker-bottom foot, 3 cases) were observed as common structural abnormalities. Mild ventriculomegaly (5 cases), oligohidramnios (1 case) and increased nuchal fold thickening (1 case) were observed as soft markers at the second trimester screening in these cases.

The mean maternal age of the CV cases was 30.5 years (range, 28- 34 years) and at the time of CV sampling the mean gestational age was 12 weeks (range, 11^{+5}- 13^{+6}). In the CV cases with soft markers all 5 fetuses had nuchal edema and other findings such as absent nasal bone (2 cases), echogenic bowel (1 cases) and echogenic intracardiac focus (1 case). These fetuses had normal karyotype. CV sampling was performed on one fetus due to detection of anencephaly, omphalocele and scoliosis as a structural abnormality. Fetal karyotype was detected as 45,X[11]/46,XX[19].

Discussion

First and second trimester fetal ultrasonographic examination as a noninvasive diagnostic tool has been employed to provide individual patient risk assessment for chromosomal abnormality.[7,11] Sonographic findings in prenatal screening are associated with fetal aneuploidy and various structural abnormalities.[4,6] Fetal sonography, when applied in the above clinical settings is cost- effective, results in a higher detection rate of trisomy 21, and is a safe procedure.[8] Although, screening with USG in a low risk population can detect many fetal malformations and may reduce perinatal mortality, it can be cause of unnecessary anxiety for parents.[2] In our study, we reviewed 179 pregnancies that underwent invasive testing for fetal karyotyping with the indication of only USG findings. None of the cases had known genetic risk or positive maternal serum screening test. We detected 12 aneuploidies as chromosomal abnormalities in the fetuses with structural abnormalities (15.78%) and 2 aneuploidies in the fetuses with soft markers (1.05%). Karyotypic abnormalities included trisomy 21 (2.23%), trisomy 18 (2.79%), trisomy 13 (0.005%) and monosomy X (0.005%). Our findings suggest that USG findings are associated with chromosome abnormalities and these are generally aneuploidies.

Major abnormalities are observed in fewer than 25% of affected fetuses in previous reports, whereas one or more soft markers may be observed in at least 50% of cases.[9] Chromosomal abnormality risk is higher among fetuses with multiple malformations (29%) as compared to those with isolated defects.[11] The importance and optimal course of action in a low-risk case with a marker on prenatal USG are controversial and not well established. Although omphalocele is a common clinical feature of trisomy 18, trisomy 13 and triploidy, it can be confused with physiologic gut herniation as a part of normal gut migration. The risk of aneuploidy is significantly increased than observed in the second trimester. Our results are consistent with these studies. In our study, among the 7 cases with omphalocele five cases had aneuploidy (Table 2). Two cases with omphalocele and structural abnormalities had normal karyotype.

Although, IUGR has been reported as an USG marker for trisomt 13 and 18 in previous studies[1], we did not observe any chromosomal abnormality in our cases with IUGR when isolated or associated with other structural abnormalities. As our study group is small, we cannot estimate the positive likelihood ratios of these findings for chromosomal abnormalities in low-risk population.

Although, fetal structural anomaly as USG finding is a well known major risk for aneuploidy, especially trisomy 21, the importance of fetal soft markers are not certain.[6,7] The most frequent chromosome abnormality, trisomy 21, is screened by ultrasonographic markers including increased nuchal fold thickness, short femur or humerus, echogenic bowel, echogenic intracardiac focus, and any major structural malformations were evaluated by fetal ultrasonography. In the current study, soft markers observed in high frequencies are increased nuchal fold thickness, choroid plexus cysts, echogenic bowel, mild ventriculomegaly and short femur. In previous studies it has been reported that most sensitive isolated soft markers for trisomy 21 include increased nuchal fold thickness, hyperechogenic bowel, shortened humerus, sortened femur and pyelectasis.[9,12] When we observed in-

creased nuchal fold thickness, hyperechogenic bowel and shortened femur as isolated markers, all cases had normal karyotype. One case with trisomy 21 had choroid plexus cysts and short femur. Some authors concluded that the presence of increased nuchal fold thickness, a structural anomaly, and a short humerus were considered sufficient to exceed the commonly accepted threshold for offering genetic diagnosis.[9,13] Some authors concluded that the presence of multiple markers (≥2) increase the risk for aneuploidy.[9,13] Eventually, if more than one marker is detected, there is a definite association with aneuploidy and offering fetal karyotyping is not a wrong decision.[13] Our finding is suggesting this management because our fetuses with abnormal karyotype had at least two or more soft markers and/or structural abnormalities except two cases with one structural abnormalitiy (Case 3 and 13).

In our study, fetal soft markers (53.07%) were observed at a higher rate than fetal structural abnormalities (42.45%), However, we detected higher aneuploidy rates in the group with structural abnormalities (15.78%) than in the group with soft markers (1.05%). We concluded that presence of one structural or two or more soft markers as fetal USG findings in low risk pregnancies increase prior aneuploidy risk. While, our results also are emphasizing an association with autosomal trisomy and fetal USG findings, it is suggested that other chromosomal abnormalities may be observed among the low risk pregnancies.[14,15]

The primary goal of routine fetal ultrasonographic screening is to detect fetal anomaly at the time when legal termination of pregnancy is an option. Genetic counseling is important when there are detected fetal USG findings. Family medical history information, maternal serum screening results, and other pertinent information must be gathered to allow for better assessment of genetic risk. Interpretation of findings and test results as well as information about any underlying disorder may be critical determinants in helping couples make decisions about the management of their pregnancy.[16] An USG marker increases the risk detected by any other screening program by 1.5 - 11 times.[13,17] On the other hand, applying the high-risk population's screening criteria to the low-risk population has resulted in unnecessarily terrifying parents and has contributed to the loss of normal fetuses through nonindicated invasive procedures.[13] Current study is performed on the cases undergoing prenatal genetic diagnosis for a fetus with abnormal USG finding. We didn't investigate the cases with a fetus having abnormal USG findings but who did not prefer prenatal diagnosis. Therefore, we cannot estimate the positive likelihood ratios of these findings for chromosomal abnormalities in low-risk population, and we cannot decide in which cases prenatal diagnosis should be strongly offered.

As a result of this study, we may conclude that, the presence or absence of soft markers can substantially modify the risk of fetal aneuploidy and one or more structural abnormalities inevitably have high risk for aneuplodies as independent factors for low-risk pregnancies.

Düşük Riskli Gebeliklerde Fetal Karyotip Anomalilerinin Saptanması Açısından Rutin Ultrasonografinin Önemi

AMAÇ: Bu çalışmada amacımız düşük anöploidi riski olan gebeliklerde fetal USG bulgularının kromozom anomalisi riskini belirlemedeki önemini değerlendirmektir.

GEREÇ VE YÖNTEM: Çalışmamızda Ocak 2002- Aralık 2008 tarihleri arasında girişimsel prenatal tanı yöntemleri uygulanan, düşük riskli gebeliklerde en sık görülen ultrasonografik "soft" belirteçler ve yapısal bozukluklar geriye dönük olarak değerlendirildi. Bu tarihler arasında prenatal tanı için başvuran hastaların 179'unda (% 9.95) fetal USG bulgusu nedeniyle sitogenetik analiz yapıldı.

BULGULAR: Hastaların 76'sında yapısal bozukluk, 95'inde "soft" belirteç, 8'inde rahim içi gelişme geriliği mevcuttu. Yapısal bozukluk olan fetusların 12 tanesinde, "soft" belirteç olanların ise 1 tanesinde anöploidi tespit edildi.

SONUÇ: "Soft" belirteçlerin varlığı veya yokluğu büyük ölçüde fetal anöploidi riskini belirleyebilir. Ancak fetusta bir veya daha fazla yapısal bozukluk saptanması, düşük riskli gebeliklerde anöploidi riskini diğer etkenlerden bağımsız olarak arttırabilir.

Anahtar Kelimeler: Anöploidi, Fetal ultrasonografi, Prenatal tanı

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