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Fetal cardiac examination can affect patients' preference on invasive tests

A new data on maternal anxiety indicated karyotyping

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Abstract

Background: Prenatal screening for aneuploidies has seen great changes over the last 2 decades. But there is still no non-invasive diagnostic test. Therefore, prenatal invasive procedures are still being routinely performed due to maternal anxiety. The association of cardiac anomalies and abnormal findings with aneuploidies has been known for a long time. This prospective study was done to evaluate abnormal fetal cardiac examination (FCE) findings on patients undergoing diagnostic invasive procedures due to maternal anxiety and to assess the predictive value of abnormal cardiac findings on abnormal karyotype.

Materials and methods: Patients who underwent prenatal diagnostic invasive tests due to maternal anxiety indication between March 2013 and September 2016 were included in this study. FCE was performed in the study group immediately prior to invasive tests. Findings of fetal cardiac examination are classified as normal, major–minor cardiac anomalies and soft markers. Fetal karyotypes were compared among groups depending on cardiac findings.

Results: One hundred eighty-two invasive procedures were performed because of maternal anxiety during this period. There were 29 abnormal findings detected on FCE. A total of 7 abnormal karyotypes were detected. FCE was abnormal in 5 of the abnormal karyotypes (71.4%). The presence of a major cardiac anomaly was most predictive for abnormal karyotype (LR+: 96,67, LR-: 0,34). No association was detected between the presence of minor cardiac anomalies and abnormal karyotype. Normal FCE appeared to be a good predictive factor for normal karyotype (LR-: 0.20).

Conclusions: This is the first study evaluating the power of early fetal cardiac examination findings on fetal aneuploidies. This study suggested that the application of fetal cardiac examination findings to genetic counseling for screening aneuploidies may be efficient on patients' preference about invasive tests. Due to the small number of abnormal findings and karyotypes detected (not the large study group), further studies on large study groups are needed to confirm these results.

Abbreviations: ACOG = American Congress of obstetricians and gynecologists, ARSA = aberrant right subclavian artery, AVSD = atrioventricular septal defect, CA = cardiac anomaly, cffDNA = cell-free fetal DNA, DS = Down syndrome, EIF = echogenic intracardiac focus, FCE = fetal cardiac examination, LR = likelihood ratio.

Keywords: aneuploidy, fetal cardiac examination, invasive karyotyping, maternal anxiety, serum screening

1. Introduction

Traditionally, advanced maternal age was considered the main risk factor for Down Syndrome (DS), and prenatal invasive procedures were routinely offered for patients >35 years.[1] Over the years, many circulating analytes have been used to give better counseling about risks for chromosomal abnormalities and certain birth defects. First and second trimester screening tests have been applied in clinical practice with improving detection rates.[2] Despite this great success, the best reports for DS detection can reach 90% with a 5% false-positive rate in first-trimester screening and can be even less in second-trimester screening.[3] After detecting the presence of cell-free fetal DNA (cffDNA) in the maternal circulation in 1997, Lo and Quake, for the first time in 2008 reported detection of fetal DS from maternal blood. This method has been used in clinical practice since 2012.[4,5] The cffDNA test provides excellent performance, for instance 98 to 99 percent of DS pregnancies are identified prenatally with less than 0.5 percent of women called screen-positive. However, it is still considered a screening test due to infrequent false-positive and false-negative results.[6] It can be placed as an intermediate step between conventional serum screening and invasive diagnostic testing. The use of cffDNA for screening women at low risk for Down syndrome is also controversial, largely because of cost. It could be implanted to screening programs only in a few countries.[7] Despite this significant development in screening strategies, maternal anxiety still presents a major indication for prenatal invasive procedures.

In the 1970's, only about 5% of pregnant women were >35 years. Recently, about 20% of pregnant women are

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>35 years old,[8] and the number of patients undergoing prenatal invasive tests has increased. Eventually, in the year 2007, the American Congress of Obstetricians and Gynecologists (ACOG) published a new bulletin for prenatal screening in which they no longer recommended invasive karyotyping for advanced maternal age. They recommended informing all pregnant women about prenatal diagnosis, screening and invasive tests, risks, benefits, and options for invasive tests for all pregnant women with an indication of maternal anxiety.[9] ACOG recommends this approach as first-line screening for most women in the general obstetric population.[7]

Population-based studies based on data from 1976 to 2004 have shown that >40% of babies with DS have a major cardiac anomaly; the most common is an atrioventricular septal defect (AVSD).[10] In addition to detection of major cardiac anomalies and cardiac septal defects, using soft markers for DS such as echogenic intracardiac focus (EIF) and aberrant right subclavian artery (ARSA), fetal cardiac examination (FCE) can give such relevant information about fetal aneuploidy diagnoses. Likelihood ratios have been determined for several soft markers, and online calculators are also available for determining DS risk using patient-specific information.[11]

The major hesitation against invasive diagnostic procedures is related to miscarriage and maternal complications. The procedure-related fetal loss rate is reported as 0.4% to 2.1% after amniocentesis, 0.7% to 1.3% and 1.4% to 1.9% after chorion villus sampling and cordocentesis respectively.[12] When patients are making a decision about invasive procedures, they straddle between the risks of procedure and fetal aneuploidy risk and consultancy is gaining importance at this point.

In this study, we aimed to estimate the prevalence of abnormal FCE findings in our population who have undergone invasive fetal karyotyping due to maternal anxiety. Also, the predictive value of normal and abnormal FCE findings for the detection of fetal aneuploidies have been evaluated, a new statistical data was introduced to counsel patients about prenatal screening for fetal aneuploidies and about their decision on invasive diagnostic tests.

2. Materials and methods

This prospective observational study was conducted in the Maternal-Fetal Medicine Unit, Department of Obstetrics and Gynecology, School of Medicine, Izmir Katip Celebi University, Izmir, Turkey, between March 2013 and September 2016. The unit is a tertiary center in the west of Turkey that treats referral patients from the region. Approval for this study was obtained by the Department of Obstetrics and Gynecology Clinic Board (registration number 28–1304). The study design was in accordance with the Helsinki Declaration (Association 2014) and confirmed with the Committee on Publication Ethics Guidelines. Written informed consent was obtained from all study participants.

Almost all of our patients are being referred by obstetricians to our Perinatal Medicine Unit from outpatient clinics of nearby hospitals in the region. We get referral patients who are applying for diagnostic prenatal invasive procedures. Consultant physicians are obstetricians and are inexperienced about early fetal cardiac examinations. Most of the patients are being evaluated for the first time during invasive procedures. Just before the invasive procedures, all patients were put through the FCE blindly; without any knowledge about the indication of invasive test. All FCEs were conducted by the same physician (Kelekci S), an expert in FCE by ultrasonography. Then all patients are grouped according to the indications of invasive procedures by another participant of the study (Ekmekci E). Referral patients who had undergone diagnostic invasive procedures due to maternal anxiety indication were included in the study. Patients who had undergone these procedures according to abnormal screening tests, abnormal major ultrasound findings, and abnormal karyotyping during a previous pregnancy were excluded from the study. Maternal and gestational ages of each patient during prenatal invasive procedures were recorded.

Patients applying for prenatal diagnostic invasive tests without any proposal from her physician, applying only according to her desire for prenatal diagnostic test are defined as maternal anxiety indicated group. This group of patients included the patients who have not applied for first trimester screening test or applied for first trimester screening and were not in the high risk group for aneuploidies according to the results (had a lower risk than the cut-off with 5% false positive rate) or did not have any detected major fetal abnormality in first trimester (cystic hygroma, exencephaly, omphalocele, increased nuchal translucency, etc.), but opted for diagnostic tests due to several reasons. The factors influencing their desire were mostly environmental factors like anomalous pregnancies at their own obstetrical history and neighborhood or advanced maternal age.

Adequate FCE is defined if fetal cardiac 4-chamber view, ventricular outflow tracts, 3 vessels, and 3 vessels-trachea views are viewed properly. Invasive procedures were performed independent of the FCE findings. The ultrasound device used was a Voluson E6 system (GE Healthcare, Milwaukee, WI) with an RAB 4 to 8 MHz transabdominal probe. FCE findings were grouped into 4 groups:

1. major cardiac anomaly (CA);
2. minor CA;
3. soft marker positivity; and
4. normal FCE.

Major CA was defined as a cardiac anomaly that must be treated and repaired surgically or is incompatible with life. Minor CA is defined as an abnormality that does not require early correction in life. Soft markers upon cardiac examination were defined as EIF and ARSA. PCR was applied to all samples with culture to have a quicker result about trisomy 21, 13 and 18. Culture results were also taken into consideration to have a precise result. Collected data were recorded on patient charts, and fetal karyotypes were recorded.

Abnormal FCE rates for abnormal karyotyped pregnancies and likelihood ratios (LR) for each finding were the primary outcome measures. Negative LR of a normal FCE was the secondary outcome measure.

The distribution of each FCE was reported using ratios. Abnormal karyotypes were reported. Statistical assessments of each group were evaluated. Diagnostic test evaluation of parametric variables for aneuploidies was carried out using MedCalc Statistical Software (MedCalc Software, Ostend, Belgium). Sensitivity and specificity ratios for each FCE finding were calculated. Positive and negative likelihood ratio values were calculated for each finding.

3. Results

In this 3.5-year period, a total of 919 invasive prenatal diagnostic procedures were performed. One hundred eighty-nine of them who had undergone invasive karyotyping due to maternal anxiety indication were included in the study (20.5%), and 730

were excluded due to other indications. The mean maternal age of patients with maternal anxiety who were karyotyped was 34.58 ± 7.37 (range: 17–46 years). The mean gestational age was 17.3 ± 1.8 (range: 16–22) weeks. Five of them underwent cordocentesis, and the other 184 underwent amniocentesis procedures. Adequate FCE was applied to the 182 patients before invasive procedures were done. Fetal position was not optimal for adequate FCE in 7 patients. A total of 29 various abnormal findings were detected on FCE. Five major cardiac anomalies were detected and they consisted of 4 atrial ventricular septal defect (AVSD) and one aorta coarctation. Also, 11 minor cardiac anomalies were detected. These were 4 cases of muscular ventricular septal defect (VSD) and 7 perimembranous VSD. A total of 13 cardiac soft markers were detected on FCE. Eleven EIF (10 of them were located in a left ventricular position, and 1 was in a right ventricular position) and 2 ARSA were detected. No findings were detected on FCE for 146 patients. A total of 7 abnormal karyotypes were detected in a total of 182 maternal anxiety-indicated patients. In the study group, a total of 7 abnormal karyotypes were detected (7/175, 4.00%). Four patients in the major CA group had abnormal karyotypes (80%). One of these karyotypes was Turner Syndrome, and the other 3 were DS. No abnormal karyotype was detected in the minor CA group. In the soft marker group, one DS with ARSA was detected. In this group, the presence of EIF was not associated with an abnormal karyotype. In the ARSA subgroup, 1 of every 2 patients had an abnormal karyotype (50%). A total of 2 abnormal karyotypes were detected in 146 normal FCE group (1.36%) and both of them were DS. Sensitivity, specificity, and positive-negative likelihood ratios of each group for abnormal karyotype are presented in Table 1. All abnormal karyotyped cases are shown in Table 2.

4. Discussion

In this study, we evaluated the efficacy of these FCE findings on screening about fetal aneuploidies. Although FCE was abnormal for 5 out of the 7 aneuploidies, it proved normal for the rest of the aneuploid fetuses. Aneuploidy cannot be excluded with a normal FCE. The most important aspect of our study is the presentation of a new statistical data that FCE may have a facilitating role in reducing patients' stress (decision making process) about invasive tests. However, this should be discussed with patients that a normal FCE cannot preclude the necessity of diagnostic tests. Some study limitations should be acknowledged including the small sample size. Despite this limitation, this represents (to the best of our knowledge) the first study on maternal anxiety-indicated karyotyping.

Detection of a major CA seems to have a strong association with abnormal karyotype, and its absence seems to show high specificity for a normal karyotype. A normal FCE had a 0.2 negative likelihood ratio for aneuploidies. But it should be noted that 3 out of 7 aneuploidies did not have any major CA. In our study group, the presence of a major CA, especially an AVSD, seemed strongly associated with an abnormal karyotype, but the presence of an isolated VSD was not associated with an abnormal karyotype in our study group. The sensitivity of early fetal echocardiography is reported at 70% and specificity at 98% if applied before 16 weeks. The most commonly diagnosed major cardiac anomalies reported in early FCE are AVSD and ventricular outflow tract anomalies.[13] In a study evaluating FCE in the first trimester (11–14 weeks), Persico et al reported 11.5% cardiac abnormalities in 867 fetuses. They explained this high first-trimester abnormality rate compared to second trimester, was due to the loss of the fetuses with cardiac anomalies in first or early second trimester.[14] In routine practice,

Table 1

Distribution of FCE findings and pooled diagnostic accuracy for each finding.

Findings	Major CA	Minor CA	Soft Markers		Normal FCE
			EIF	ARSA	
Frequency (n)	5	11	11	2	153
Abnormal Karyotype Ratio (%)	4/5 (80%)	0/11 (0%)	0/11 (0%)	1/2 (50%)	2/146 (1.36%)
Sensitivity (%)	66.67	0	0	33.33	–
Specificity (%)	99.31	92.90	92.90	99.31	–
LR+	96.67	0	0	48.33	–
LR−	0.34	1.08	1.08	0.67	0.20

−, indicates not mentioned.

ARSA = aberrant right subclavian artery, CA = cardiac anomaly, EIF = echogenic intracardiac focus, FCE = fetal cardiac examination, LR- = negative likelihood ratio, LR+ = positive likelihood ratio.

Table 2

Distribution of abnormal karyotypes and FCE findings.

	Gestational Age	Abnormal Finding	Maternal Age	Karyotype
Case 1	16 weeks	AVSD	43	Down Syndrome
Case 2	16 weeks	AVSD	40	Down Syndrome
Case 3	19 weeks	Aort Coarctation	24	Turner Syndrome
Case 4	17 weeks	AVSD	37	Down Syndrome
Case 5	17 weeks	ARSA	32	Down Syndrome
Case 6	22 weeks	–	17	Down Syndrome
Case 7	18 weeks	–	41	Down Syndrome

−, indicates not mentioned.

AVSD = atrioventricular septal defect, ARSA = aberrant right subclavian artery.

FCE is performed in the second trimester (18–22 weeks) for high-risk patients or when there is a suspicion for CA in the second trimester.

The majority of fetal CAs occurred in low-risk populations.[15] In this study, FCE's were performed between 16 and 22 gestational weeks immediately prior to invasive procedures for low-risk patients. It is because the mean gestational age in our study group was 17.3±1.8 weeks, most of our FCE's can be defined as early fetal cardiac examinations. High AVSD and VSD detection rates can be associated with early detection of abnormalities. In a large study, the prevalence of EIF was reported as 3.2% and isolated EIF in the fetal heart in the mid-trimester of pregnancy was reported not to be associated with abnormal karyotype in low-risk populations.[16] In our study group; no abnormal karyotype was detected in 11 EIF fetuses. In postnatal series, the prevalence of cardiac anomalies are reported as 40% to 50% for trisomy 21, 25% to 35% for Turner Syndrome, and >80% for trisomy 13 and 18[17,18]. In an epidemiologic study in 1989, 13% of 2012 live births associated with cardiac abnormalities were reported to have chromosomal abnormalities. In this study, the major aneuploidy was DS with 10.4% rate, and others were <1%.[17] The differences in prenatal and postnatal chromosomal abnormality rates were due to high stillbirth rate in fetuses with CA, and the stillbirth rate was reported as 30% for trisomy 21, 1.42% for trisomy 13.68%, for trisomy 18, and 75% for Turner Syndrome.[19] In our study group, abnormal cardiac findings were detected in 4 out of 6 DS fetuses (66.6%). But that should be remarked that no abnormal findings were detected on FCE in 2 fetuses with DS.

In our study group, the total chromosomal abnormality rate was 3.84%. This rather high aneuploidy rate may be arguable. We think that was a statistical clustering, because there was a clustering in both aneuploidies and cardiac anomaly rate. Cardiac anomaly rate was also higher than the general. Also, this clustering did not have any negative effect on study design. On the contrary, the aim of the study was to evaluate the cardiac anomalies during invasive procedures so that we could have a certain result earlier. In another recent study from the Netherlands, they reported the positive predictive value of referrals for advanced maternal age only (>36 years), 1.0% and 1.8% for amniocentesis and chorionic villous biopsy for DS, respectively.[20] In our study, the maternal anxiety indication group consisted of patients of all age groups, not just advanced ages. The mean age in abnormal karyotype group was 33.42± 3.67, and 4 of the 7 patients were >36 years. The advanced maternal age and earlier detection of aneuploidies may be a factor for clustering also.

Chorionic villous sampling was not the preferred procedure by patients. Amniocentesis seems to be the preferred one due to lower complication rates. This preference seems logical if the study population is considered. Also, the gestational ages at patient admission were more appropriate for amniocentesis.

In our study 5 major CAs were detected. Three out of 4 AVSDs were associated with DS (75%), and 1 aorta coarctation was associated with Turner Syndrome. One AVSD was not associated with abnormal karyotype. In a large prevalence study in Europe, CAs were the most frequent congenital anomalies in DS fetuses and 30% were AVSD, atrial septum defect was 25%, 22% ventricular septal defect, 5% patent ductus arteriosus, 5% aortic coarctation, and tetralogy of Fallot was 3%.[21] If the antenatal diagnosis of AVSD was isolated, 58% was associated with DS.[22] Prenatally, detection of aortic coarctation has been reported to have a 35.1% association with abnormal karyotype and about 40% of them had Turner Syndrome.[23] In our study group, the presence of an isolated VSD was not associated with an abnormal karyotype. High detection rates and low association with aneuploidies may be due to the closure of small defects in the mid second trimester. Detection of VSD in early second trimester did not seem to be related to abnormal karyotype. The prevalence of ARSA in DS fetuses was reported as 23.6%, whereas 1.02% was reported in euploid fetuses. There is insufficient evidence to recommend fetal karyotyping in cases with isolated ARSA.[24] In our study, the prevalence of isolated ARSA was 1.14% (2/175). Although this sample size was too small to recommend karyotyping for isolated cases, we feel that the presence of ARSA will give valuable information for counseling.

The main innovation of our results is the development of a statistical data that may be used to counsel the patients about diagnostic prenatal invasive tests. Negative and positive likelihood ratios may be used to recalculate the patient specific fetal DS risk after screening tests and/or sonography. Similar objective methods are described to constitute a patient specific risk by computer software to evaluate the fetal status, like cardiotocographic indices.[25,26]

In conclusion, this is the first study evaluates the efficacy of early FCE findings and describes predictive values to be used for genetic counseling. Nowadays, women are more anxious about having chromosomal anomalous babies because of the delay of pregnancies to older ages. In addition to screening tests, genetic counseling with FCE findings may reduce patients' anxiety. A normal FCE had a 0.2 negative likelihood ratio for aneuploidies. Counseling in conjunction with the implementation of FCE findings, especially for this group, may be sufficient for patients' preferences about invasive tests. However, that should be particularly mentioned that our findings can be useful only for counseling. Normal FCE cannot conclude a normal fetal karyotype. Abnormal and normal FCE finding results can be used for counseling with patients' preference about invasive tests with positive-negative likelihood ratios of each. FCE findings can be useful in terms of justifying the risk of a procedure. Additional and more extensive studies with larger study population are required in this field.

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References

[1] Ogilvie CM. Prenatal diagnosis for chromosome abnormalities: past, present and future. Pathole Biol 2003;51:156–60.

[2] Canick J, Lambert-Messerlian G, Palomaki G, et al. First and Second Trimester Evaluation of Risk (FASTER) trial research consortium. Comparison of serum markers in first-trimester down syndrome screening. Obstet Gynecol 2006;108:1192–9.

[3] Wapner R, Thom E, Simpson JL, et al. First-trimester screening for trisomies 21 and 18. N Engl J Med 2003;349:1405–13.

[4] Lo YD, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485–7.

[5] Fan HC, Blumenfeld YJ, Chitkara U, et al. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci USA 2008;105:16266–71.

[6] Mackie F, Hemming K, Allen S, et al. The accuracy of cell-free fetal DNA-based non-invasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis. BJOG: Int J ObstetrGynaecol 2017;124:32–46.

[7] Society for Maternal-Fetal Medicine (SMFM) Publications Committee#36: Prenatal aneuploidy screening using cell-free DNA. Am J Obstetr Gynecol 2015;212:711–6.

[8] Hodges RJ, Wallace EM. Testing for Down syndrome in the older woman: a risky business? Austr N Zeal J Obstetr Gynecol 2005;45:486–8.

[9] ACoP B. ACOG Practice Bulletin No. 77: screening for fetal chromosomal abnormalities. Obstet Gynecol 2007; 109:217–27.

[10] de Rubens Figueroa J, del Pozzo Magaña B, Hach JLP, et al. Heart malformations in children with Down syndrome. Rev Esp Cardiol 2003;56:894–9.

[11] Aagaard-Tillery KM, Malone FD, Nyberg DA, et al. Role of second-trimester genetic sonography after Down syndrome screening. Obstet Gynecol 2009;114:1189.

[12] Tongsong T, Wanapirak C, Kunavikatikul C, et al. Fetal loss rate associated with cordocentesis at midgestation. Am J Obstet Gynecol 2001;184:719–23.

[13] McAuliffe FM, Trines J, Nield LE, et al. Early fetal echocardiography—a reliable prenatal diagnosis tool. Am J Obstet Gynecol 2005;193:1253–9.

[14] Persico N, Moratalla J, Lombardi C, et al. Fetal echocardiography at 11-13 weeks by transabdominal high-frequency ultrasound. Ultrasound Obstet Gynecol 2011;37:296–301.

[15] Sharland G. Routine fetal cardiac screening: what are we doing and what should we do? Prenatal diagnosis 2004;24:1123–9.

[16] Merati R, Lovotti M, Norchi S, et al. Prevalence of fetal left ventricular hyperechogenic foci in a low risk population. BJOG: Int J Obstetr Gynaecol 1996;103:1102–4.

[17] Ferencz C, Neill CA, Boughman JA, et al. Congenital cardiovascular malformations associated with chromosome abnormalities: an epidemiologic study. J Pediatr 1989;114:79–86.

[18] Embleton N, Wyllie J, Wright M, et al. Natural history of trisomy 18. Arch Dis Child Fetal Neonatal Ed 1996;75:F38–41.

[19] Hook EB. Chromosome abnormalities and spontaneous fetal death following amniocentesis: further data and associations with maternal age. Am J Hum Genet 1983;35:110.

[20] Siljee JE, Knegt AC, Knapen MF, et al. Positive predictive values for detection of trisomies 21, 18 and 13 and termination of pregnancy rates after referral for advanced maternal age, first trimester combined test or ultrasound abnormalities in a national screening programme (2007-2009). Prenat Diagn 2014;34:259–64.

[21] Stoll C, Dott B, Alembik Y, et al. Associated congenital anomalies among cases with Down syndrome. Eur J Med Gene 2015;58:674–80.

[22] Delisle M-FN, Sandor GG, Tessier F, et al. Outcome of fetuses diagnosed with atrioventricular septal defect. Obstet Gynecol 1999; 94:763–7.

[23] Paladini D, Volpe P, Russo M, et al. Aortic coarctation: prognostic indicators of survival in the fetus. Heart 2004;90:1348–9.

[24] Scala C, Leone Roberti Maggiore U, Candiani M, et al. Aberrant right subclavian artery in fetuses with Down syndrome: a systematic review and meta-analysis. Ultrasound Obstetr Gynecol 2015;46:266–76.

[25] Romano M, Bifulco P, Ruffo M, et al. Software for computerised analysis of cardiotocographic traces. Comput Methods Programs Biomed 2016; 124:121–37.

[26] Romano M, Iuppariello L, Ponsiglione AM, et al. Frequency and time domain analysis of foetal heart rate variability with traditional indexes: a critical survey. Comput Math Methods Med 2016;2016:9585431.