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RESEARCH

Cytogenetic evaluation of 661 prenatal samples

661 prenatal örneğin sitogenetik değerlendirmesi

Seda Eren Keskin[1], Buket Doğruoğlu[1], Zeynep İlkay[1], Deniz Sünnetçi Akkoyunlu[1], Naci Çine[1], Hakan Savlı[1], Yasemin Doğan[1], Gülseren Yücesoy[1]

[1]Kocaeli University, Kocaeli, Türkiye

Abstract

Purpose: Fetal karyotyping is commonly used to detect chromosomal abnormalities in high-risk pregnancies. Our study is intended to evaluate the results of fetal karyotyping performed in our laboratory for six years and to determine the frequency of chromosomal abnormalities, thus revealing their clinical significance.

Materials and Methods: The cytogenetic results of 661 prenatal samples with an indication for invasive prenatal procedures (amniocentesis, cordocentesis) who had a chromosome analysis and FISH testing between February 2013 and March 2019 were analyzed in our study.

Results: A total of 72 (10.8%) abnormal fetal karyotypes were observed in the study group. Trisomy 21 was the most common numerical aberration (29%, n = 23), followed by trisomy 18 (16%, n = 13), trisomy 13 (2.6%, n = 2), triploid (2.6%, n = 2), sex chromosome aneuploidies (5.2%, n = 4), and rare mosaic autosomal aneuploidies (2.6%, n = 2). Inversions (16%, n = 13), inherited translocations (7.8%, n = 6), unbalanced/de novo translocations (6.5%, n = 5), deletions (5.2%, n = 4), additional chromosomes (1.3%, n = 1), isochromosomes (1.3%, n = 1), and derivative chromosomes (1.3%, n = 1) were identified as structural abnormalities. Of the 18 cases that underwent FISH testing, trisomy 18 was detected in 1 case and tetrasomy 12p was detected in 1 case.

Conclusion: Fetal karyotyping is still an effective and valuable method in the diagnosis of fetal anomalies and provision of effective genetic counseling. In addition, fetal karyotyping should be supported by complementary methods and advanced technologies for accurate and rapid prenatal genetic diagnosis.

Keywords:. Prenatal diagnosis, amniocentesis, cordocentesis, cytogenetic analysis, karyotyping

Öz

Amaç: Fetal karyotipleme, yüksek riskli gebeliklerde kromozomal anomalilerin belirlenmesinde yaygın olarak kullanılmaktadır. Çalışmamızda, laboratuvarımızda altı yıl boyunca gerçekleştirilen fetal karyotipleme sonuçlarını değerlendirmek ve kromozomal anormalliklerin sıklığını belirleyerek klinik önemini ortaya koymak amaçlanmaktadır.

Gereç ve Yöntem: Şubat 2013 ve Mart 2019 arasında kromozom analizi ve FISH testi yaptıran ve invaziv prenatal işlem (amniyosentez, kordosentez) endikasyonu olan 661 prenatal numunenin sitogenetik sonuçları incelenmiştir.

Bulgular: Çalışma grubunda toplam 72 (%10,8) anormal fetal karyotip gözlenmiştir. Trizomi 21 en yaygın sayısal aberasyon olup (%29, n = 23), bunu trizomi 18 (%16, n = 13), trizomi 13 (%2,6, n = 2), triploidi (%2,6, n = 2), cinsiyet kromozomu anöploidileri (%5,2, n = 4) ve nadir mozaik otozomal anöploidiler (%2,6, n = 2) izlemiştir. Yapısal anormallikler olarak inversiyonlar (%16, n = 13), kalıtsal translokasyonlar (%7,8, n = 6), dengesiz/de novo translokasyonlar (%6,5, n = 5), delesyonlar (%5,2, n = 4), ilave kromozomlar (%1,3, n = 1), izokromozomlar (%1,3, n = 1) ve derivatif kromozomlar (%1,3, n = 1) tespit edilmiştir. FISH testi yapılan 18 vakanın birinde trizomi 18 ve birinde tetrazomi 12p saptanmıştır.

Sonuç: Sonuçlarımız, fetal karyotiplemenin fetal anomalilerin tanısında ve etkin genetik danışmanlığın sağlanmasında hala etkili ve değerli bir yöntem olduğunu göstermektedir. Ayrıca doğru ve hızlı prenatal genetik tanı için fetal karyotiplemenin tamamlayıcı yöntemler ve ileri teknolojilerle desteklenmesi gerekmektedir.

Anahtar kelimeler: Prenatal tanı, amniyosentez, kordosentez, sitogenetik analiz, karyotip

Address for Correspondence: Zeynep İlkay, Kocaeli University, Faculty of Medicine, Department of Medical Genetics, Kocaeli, Türkiye E-mail: zeynepp85@gmail.com
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INTRODUCTION

Chromosomal abnormalities (CAs) are the most common underlying cause of many congenital anomalies, and they occur in about 1 in 150 live births. CAs cover aneuploidies, duplications, deletions, and translocations. Trisomy 21, or Down syndrome, is the most common CA affecting 1 in every 800 live births. Trisomy 13, trisomy 18, and sex chromosomal aneuploidies have a lower incidence[1].

The main indications for prenatal diagnosis suggesting CAs are considered to be advanced maternal age, abnormal USG findings, increased risk of screening tests, and a family history of CAs[2].Invasive and non-invasive methods are used for the diagnosis of chromosomal diseases in the prenatal period. Non-invasive tests include maternal serum biochemical marker screening and cell-free DNA (cff-DNA) screening to help identify high-risk pregnancies. Non-invasive prenatal testing (NIPT), known as the cff-DNA based screening method, is highly sensitive to placenta-derived CAs. Abnormal screening test results require confirmational genetic analysis through invasive procedures such as amniocentesis (AS) or cordocentesis (CS)[3].

Karyotyping is the most extensively used procedure for detecting fetal CAs, with a 99.5% accuracy[4]. Molecular testing methods are also available for the detection of genetic abnormalities, such as submicroscobic deletions and duplications with microarray technology, and Fluorescent In Situ Hybridization (FISH) for autosomal aneuploidies or microdeletions and microduplications. The FISH test offers rapid analysis of both cultured and uncultured materials. Microarrays are capable of high-resolution analysis and they are frequently used, particularly in cases of abnormal USG[5].

Mosaicism is the presence of multiple chromosomally distinct cell lines in an individual from the same zygote. Prenatal cytogenetic analysis detects three levels of mosaicism in amniotic fluid in vitro: level I, which is a culture artifact; level II, where two or more cells with the same defect are disseminated in distinct cultures; level III, which requires resampling. Resampling is necessary to verify mosaicisms[6].

A genetic counseling session is an integral part of the prenatal diagnosis of CAs. During the genetic counseling session, patients should be informed about the contributions, limitations, and risks of screening and diagnostic tests[7].

In this study, we present a single-center experience of structural and numerical CAs detected during prenatal diagnosis during six years. Our data may provide important information for proper genetic counseling and development of more effective genetic strategies. This study confirms that chromosome analysis is still the most valid method for the detection of fetal chromosomal anomalies.

MATERIALS AND METHODS

Sample

In this study, we conducted a retrospective examination of fetal karyotyping with AS and CS for six years. AS, performed in the second trimester (between the 16th and 24th weeks), and CS, performed after the 18th week, were included in the study. However, samples without a clinical indication for an invasive procedure (isolated polyhydramnios) and CS materials with maternal blood contamination were excluded from the study. Considering these criteria, we reviewed the clinical indications and cytogenetic results of 602 amniotic fluid (AF) samples and 59 cord blood (CB) samples received between February 2013 and March 2019 at our laboratory.

Perinatology Division, Department of Obstetrics and Gynecology, Kocaeli University Medical Faculty, performed invasive procedures (AS and CS). The Department of Medical Genetics of the Medical Faculty at Kocaeli University analyzed the chromosomes of the patients. A written informed consent was obtained from each patient for the publication of their genetic test results in scientific studies.

Ethics committee approval was obtained from Kocaeli University Non-Interventional Research Ethics Committee with a decision dated June 16, 2023 and numbered 2023/198.

Procedure

IndicationsPregnant women who appeared to be in need of invasive prenatal procedures referred to our department for pre-test genetic counseling. All the patients were informed about the content of genetic testing during pre-test counseling. Clinical indications for prenatal genetic testing with chromosome analyses were grouped as follows: High serum screening test risk, abnormal USG findings, advanced maternal age, previous child with a chromosome abnormality, non-invasive prenatal genetic test

confirmation, maternal anxiety, mosaicism confirmation, previous fetus with an anomaly, and family history with chromosome abnormality[8].

Chromosome analysis

Samples from AS and CS were cultured according to standard procedures. For CS, using the Apt test (alkaline hemoglobin), the risk of maternal contamination was excluded. For cytogenetic analyses, a minimum of 20 metaphases from two separate culture flasks were analyzed for each sample. Karyotype results were reported according to International System for Human Cytogenetic Nomenclature (ISCN 2013)[9]. According to Gardner and Sutherland, heterochromatin variants and satellite variants were not reported as chromosome abnormalities[10].

FISH

FISH was applied to a total of 19 samples (18 cases), with the referrals as follows: culture failure (n = 8), insufficient number of metaphases (metaphase count <20, n = 4), sonographic cardiac defect (n = 4), and extra chromosomal material identification (n = 2). Cultured samples were tested using probes according to the manufacturer's protocol. Fifty-one hundred nuclei were counted and analyzed for each probe or region. Table 3 lists the details of probes, tested regions, indications, and results.

Statistical analysis

The study used G Power 3.1.9.7 program for post hoc power analysis with 661 participants, resulting in an effect size of 0.25, a type 1 error of 0.05, and a power of 0.99. Statistical calculations were performed with IBM SPSS version 21. Continuous variables were shown as mean, and categorical variables were shown as numbers and percentages. The Kolmogorov-Smirnov test assessed data normality, chi-square analysis compared categorical variables, and a significance level of $p<0.05$ was utilized.

RESULTS

In our laboratory, 661 prenatal samples were analyzed for chromosomes between February 2013 and March 2019. At the time of the invasive procedure, the mean age of the mothers was 32.6 ± 6.78 (range: 15 - 48 years).

Table 1. Detection rates of abnormal karyotypes in clinical indications

Indication	Number of Cases (%)			Abnormal Cases (%)		
	AS	**CS**	**TOTAL**	**AS**	**CS**	**TOTAL**
High Serum Screening test risk	360(60%)	8(13%)	368(55%)	28(7.8%)	2(25%)	30(8%)
Abnormal USG findings	153(25%)	47(77%)	200(29%)	25(16%)	3(6.5%)	28(14%)
Advanced Maternal Age	72(12%)	2(3.3%)	74(11%)	9(12.5%)	1(50%)	10(13%)
Previous child with chromosome abnormality	6(1%)	-	6(1%)	-	-	-
Non-invasive prenatal genetic test confirmation	4(0.6%)	-	4(0.6%)	4(100%)	-	4(100%)
Maternal anxiety	4(0.5%)	-	3(0.5%)	-	-	-
Mosaicism Confirmation	1(0.1%)	1(0.1%)	2 (0.3%)	-	-	-
Previous fetus with anomaly	1(0.1%)	1(0.1%)	2(0.3%)	-	-	-
Family history with chromosome abnormality	1(0.1%)	-	1(0.1%)	-	-	-
TOTAL	602	59	**661**	66	6	**72**

AS: Amniocentesis, CS: Cordocentesis

The most common reason for an invasive procedure was a high serum screening test risk (n = 368, 55%), followed by abnormal ultrasound results (n = 200, 29%), advanced maternal age (n = 74, 11%), and a previous child with a CA (n = 6, 1%). Other rare indications below 1% included confirmation of a non-invasive prenatal genetic test (n = 4, 0.6%), maternal anxiety (n = 4, 0.5%), confirmation of mosaicism (n = 2, 0.3%), a previous infant with an anomaly (n = 2, 0.3%), and a family history of a CA (n = 1, 0.1%), respectively. Table 1 details the clinical indications for each invasive procedure.

Our rate of cultural success was 96.4% (n = 580) for amniocentesis material. In 10.8% of the cases, an abnormal karyotype was identified. The CAs were classified into numerical and structural categories. Trisomy 21 was the most prevalent numerical aberration (29%, n = 23), followed by trisomy 18 (16%, n = 13), trisomy 13 (2.6%, n = 2), triploidy (2.6%, n = 2), sex chromosome aneuploidies (5.2%, n = 4), and rare mosaic autosomal aneuploidies (2.6%, n = 2). As structural aberrations, the following were identified: inversions (16%, n = 13), inherited translocations (7.8%, n = 6), unbalanced/de novo translocations (6.5%, n = 5), deletions (5.2%, n = 4), additional chromosomes (1.3%, n = 1), isochromosomes (1.3%, n = 1), and derivative chromosomes (1.3%, n = 1). In our group, one of the rare referral reasons for the invasive procedure was NIPT result confirmation with a 0.6% (n = 4) rate that was performed by another laboratory. Trisomy 21 was detected in three cases, and trisomy 18 in one. All these cases were confirmed by karyotyping, consistent with the NIPT results. The details of all chromosomal aberrations detected by chromosome analysis are listed in Table 2. FISH. Among the 19 samples that had FISH analysis, one sample exhibited trisomy 18, and two samples showed tetrasomy 12p.

Table 2. Incidence of each chromosome abnormality according to indications

Type of Abnormality	HSSTR	AbUSG	AMA	CWCA	NIPTC	TOTAL
Numerical	17	19	5		4	46(59%)
Trisomy 21	9	8	3	-	3	23(29%)
Trisomy 18	5	5	2	-	1	13(16%)
Trisomy 13	-	2	-	-	-	2(2.6%)
69,XXX	1	1	-	-	-	2(2.6%)
Monosomy X	-	3	-	-	-	3(3.9%)
Trisomy X	1	-	-	-	-	1(1.3%)
Mosaic rare autosomal aneuploidy (trisomy 5, trisomy 10)	2	-	-	-	-	2(2.6%)
Structural						31(41%)
De novo/unbalanced	5	4	2	1	-	12(38%)
Deletion 5p15,18p,16p13.1(mos), 13q21(mos)	2	1	-	1	-	4(5.2%)
Addition add(7p)	-	-	1	-	-	1(1.3%)
Isochromosome mos tetrasomy 12p	-	1	-	-	-	1(1.3%)
Translocation t(4;12)(q31;q13) der(15)t(15:16)(q10;q11.2)mat mos t(6;21)(q14;11,2) der(13)(6;13)t(6;13)(q23.?;q32.?)mat t(18;21)(q11;q10),+18	2	2	1	-	-	5(6.5%)
Derivative Chr Chr8	1			-	-	1(1.3%)
Balanced/Parental	10	8	2	-	-	19(62%)
Inversion 9p9q,4pq21, 16pterq11(mos)	8	4	2	-	-	13(16%)
Translocation t(3;6)(p13;q27)mat t(3;16)(q11;q22)mat rob(14;21),(13;14) t(1;7)(q11;q11)pat	2	4	-	-	-	6(7.8%)
	32	31	8	1	4	76

AbUSG (abnormal USG findings), AMA (advanced maternal age), HSSTR (high serum screening test risk), CWCA (child with a chromosome abnormality), NIPTC (non-invasive prenatal test confirmation),

Table 3. FISH results of tested cases

Probe/Tested Regions	Sample	Indication	Karyotype	FISH Result
Cytocell Aneucyte Probe 13q14.2, 18p11.1-q11.1, 21q22.13, Xp11.1-q11.1, Y p11.1-q11.1				
	AF	HSSTR	47,XY,+18[8]	%100 Trisomy 18
	AF	HSSTR	-	Normal
	AF	AbUSG	-	Normal
	AF	AMA	-	Normal
	AF	HSSTR	-	Normal
	AF	HSSTR	46,XY[6]	Normal
	AF	AbUSG	-	Normal
	AF	HSSTR	-	Normal
	AF	HSSTR	-	Normal
	AF	HSSTR	46,XX[3]	Normal
	AF	HSSTR	46,XX[9]	Normal
	AF	AbUSG	-	Normal
CytoCell DiGeorge TBX1 and 22q13.3 Deletion, CytoCell DiGeorge/VCFS N25 and 22q13.3 Deletion CytoCell DiGeorge/ VCFS TUPLE1 and 22q13.3 Deletion Probe Combination, 22q11.2 22q13.3				
	AF	AbUSG	46,XY[20]	Normal
	AF	AbUSG	46,XX[20]	Normal
	CB	AbUSG	46,XY[20]	Normal
	CB	AbUSG	46,XY[20]	Normal
CytoCell TEL/AML1 (ETV6/RUNX1) Translocation, Dual Fusion, 12p13.2, 21q22.12				
	AF CB	AbUSG	47, XY,+mar[8]/46,XY[54] Final Karyotype: 47, XY,+i(12p)[8]/46,XY[54] 46,XY[100]	% 14 Tetrasomy 12p % 4 Tetrasomy 12p

AF (Amniotic fluid), AbUSG (Abnormal USG findings), AMA (Advanced maternal age), CB (Cord blood), HSSTR (High serum screening test risk).

Table 4. Classification of USG findings with abnormal karyotype

	n	Abnormal karyotype(%)		n	Abnormal karyotype
Cardiovascular System	**63**	**17(27%)**	**Genitourinary System**	**20**	**4(20%)**
Perimembranous VSD	2	1(50%)	Pyelectasis	13	2(15%)
Fallot tetralogy	6	2(33%)	Megacystis	4	1(25%)
AVSD	6	3(50%)	Unilateral dysplastic kidney	1	1(100%)
Double outlet ventricle	5	2(40%)	**Neck/Body Fluids**	**48**	**13(16%)**
Echogenic intracardiac focus	9	3(33%)	Non immune hydrops	5	2(40%)
Hypoplastic left heart	5	1(20%)	Hydrops	10	1(10%)
Uterine artery notch	2	1(50%)	Increased NT	9	3(33%)
Pulmonary stenosis	2	1(50%)	Acid	2	1(50%)
Pulmonary atresia	4	1(25%)	Pleural effusions	5	1(20%)
Central Nervous System	**85**	**11(13%)**	Cystic hygroma	14	5(35%)
Cerebellum hypoplasia	3	1(33%)	**Neural Tube Defects**	**24**	**1(4%)**
Corpus collosum agenesia	7	1(14%)	Meningocele	1	1(100%)
Ventriculomegaly	16	4(25%)	**Gastrointestinal System**	48	5(10%)
Hydrocephalus	14	1(7%)	Hyperechogenic bowel	27	2(7%)
Colpa cephalic lateral ventricle	1	1(100%)	Calcified focus on the gastric cavity	1	1(100%)
Brachycephaly	1	1(100%)	Omphalocele	10	1(10%)
Choroid plexus cyst	17	2(13%)	Fetal small stomach	4	1(25%)
Facial features	**10**	**2(20%)**			
Micrognatia	3	1(33%)	**Other**	**10**	**3(33%)**
Hypertelorism	3	1(33%)	Intrauterine growth retardation	6	1(16%)
Musculoskeletal System	39	2(5%)	Single umbilical artery	4	2(50%)
Extremity deformity	4	1(25%)	Diapharmatic hernia	11	1(9%)
Bilateral rocker bottom feet	3	1(33%)	**TOTAL**		**57**

There were 256 aberrant USG results among all patients, regardless of the reason for referral. There were 58 isolated or multiple minor or major ultrasound markers for aneuploidies. Twenty-nine percent (n = 192) of the patients exhibited major sonographic markers for aneuploidy, and 13% (n = 85) exhibited minor sonographic markers for aneuploidy. Table 4 lists the systematic classification of markers with an abnormal karyotype.

DISCUSSION

The definition of CAs in fetuses with fetal disorders and high risk for aneuploidies requires prenatal sampling with invasive procedures[11]. Trisomies of chromosome 21, 18, 13, monosomy X, and other sex chromosome aneuploidies can account for approximately 95% of the CAs in the newborns. Therefore, chromosome analysis is essential for detecting aneuploidies and other structural chromosomal anomalies[12]. Cell culture success is critical for analyzing chromosomes from amniocentesis material. The success rate of our cell culture was 96.4% (n = 22). Taşdemir et al.[13] reported the rate of culture success as 95% and Türkyılmaz et al.[12] reported it as 97%, both of which were close to our rate. FISH was applied to 8 samples with culture failure, and no aneuploidy (chr. 13, 18, 21, X and Y) was detected. Resampling was advised for 14 samples with insufficient cell counts for hybridization. Also,

FISH was utilized to exclude low-level mosaicism (chr. 13,18, 21, X, and Y) on 4 samples in which an adequate number of metaphases could not be examined. FISH method is a necessary complementary method in prenatal diagnosis[14].

Previous studies of fetal karyotyping by amniocentesis and cordocentesis have revealed the incidence of CAs to be between 2.9% and 20.9% [2,8,12-29]. In our group, the overall prevalence of CAs detected by chromosome analysis was 10.8%, which ranged from 0% to 100% in different indications [Table 5]. Our rate was consistent with the rates of 9.8% in the study by Andrew et al.[24], 9.8% in the study by Younesi et al.[27] and, 11,1% in the study by Durmaz et al.[25]

In our study, the predominant CAs were autosomal aneuploidies, including trisomy 21, trisomy 18, and trisomy 13, representing 48% of total CAs. Similar frequencies were reported by Steth et al.[19] and Zhang et al.[23] at the rates of 46% and 49%, respectively. In this study, trisomy 21 (29%) was the most common chromosomal aberration among all the cases, which is consistent with the fact that trisomy 21 is the most frequent CA in prenatal cases. Xiao et al.[18] also reported that trisomy 21 was the leading chromosome abnormality and accounted for 25% of 12.365 specimens of AF. Similar reports from Durmaz et al.[25], and Lopez et al.[28] found the trisomy 21 detection rate at 31% and 51%, respectively. The wide range of detection rates may occur due to the different distribution of indications for each study.

Pallister-Killian syndrome is a rare genetic disorder characterized by tissue-limited mosaicism in isochromosome 12p. In prenatal terms, the diaphragmatic hernia is a major criterion in second-trimester ultrasonography. The prenatal diagnosis of PKS is still complex due to the similarity of tetrasomy 12p and tetrasomy 21q and the unequable mosaicism level. Mosaicism is higher in fibroblast-derived tissues like amniocytes than in lymphocytes, which rapidly grow[30]. In one patient, we detected a mosaic marker chromosome similar to tetrasomy 21q on amniocentesis material. However, the coexistence of diaphragmatic hernia and mosaic chromosome aberrations suggested PKS. We recommended CS for further analysis. FISH was performed on both the AF samples and the CB samples. Chromosome analysis of fetal blood showed a normal karyotype. FISH analysis confirmed that the mosaic marker structure was of 12p origin. Tetrasomy 12p was observed at a rate of 14% in amniotic cells and 4% in blood. Mosaicism was higher in amnion cells than in blood[30], a result which was consistent with the literature.

Table 5. Distribution of indications and chromosome anomaly frequencies from similar reports

Reference Country	n	AMA (%)	HSSTR (%)	AbUSG (%)	Abnormal Karyotype (%)	Tri 21 (%)	Tri 18 (%)	Tri 13 (%)	Sex CA (%)	SCA (%)
Türkyılmaz A et al., 2007, Turkey [12]	481	14%	34%	25%	7%	39%	0%	0%	14%	29%
Şimşek S et al., 2011, Turkey [15]	649	13%	36%	28%	17%	NR	NR	NR	NR	NR
Taşdemir Ş et al, 2014, Turkey [13]	1429	40.4%	38.9%	17.3%	4.3%	62.9%	10%	9%	3.2%	19%
An N et al, 2015, China [16]	2500	15.41%	69.56 %	3.48%	4%	37%	1.1%	0.9%	5.5%	37%
Soler MI et al, 2015, Spain [17]	29883	30%	44.1%	6.2%	2.9%	37%	7%	2%	13.2%	26.2%

Xiao H et al., 2015, China [18]	123655	34.53%	40.18%	8.18%	3.46%	25%	9.6%	1.4%	10%	53.5%
Sheth F et al, 2015, India [19]	1728	32%	51%	13%	7.2%	36%	8%	2%	12%	45.6%
Nishiyama M. et al, 2015, Japanese [20]	28983	54.7%	18.4%	14.1%	6%	43.5%	17.3%	3.1%	11.6%	18.4%
Acar A et al., 2016, Turkey [21]	3721	35.8%	45.1%	15.8%	3.6%	48%	9.1%	3.8%	6%	19.1%
Tao H et al., 2017, China [22]	4761	39.1%	46.8%	4.4%	2.88%	59%	16%	0%	11%	10.9%
Zhang S et al., 2017, China [23]	5328	0%	0%	100%	4.2%	76.5%	29.1%	7.1%	16.5%	23.5%
Zhang S et al., 2017, China [8]	40208	29.8%	43.6%	13.25%	3.36%	37.44%	11.6%	1.11%	10.23%	36.9%
Andrew C et al., 2018, India [24]	257	31%	51%	26%	9.8%	44%	20%	8%	4%	20%
Li H et al., 2019, China [2]	4206	NR	NR	NR	8.54%	48.32%	14.25%	0%	10.61%	10%
Durmaz B et al., 2021, Turkey [25]	9297	48.2%	25.7%	11.1%	5.7%	31%	7.8%	2.4%	10.6%	39%
Bozdoğan TS et al., 2021, Turkey [26]	2843	NR	NR	NR	9.11%	42%	21%	6.9%	1.59%	21.9%
Younesi S et al., 2021, Iran [27]	15,401	11.3%	72.9%	14.5%	9.8%	54%	7%	2.9%	5.29%	12%
Lopez JJ et al., 2021, Colombia [28]	3961	NR	NR	NR	20.9%	51.7%	18.6%	8.1%	11.2%	5.1%
Moczulska H et al., 2023, Poland [29]	2169	NR	NR	NR	9.4%	45%	15%	8.7%	9.7%	18%

AbUSG (abnormal USG findings), AMA (advanced maternal age), HSSTR (high serum screening test risk), NR: Not Reported, SCA (Structural Chromosome Abnormalilities), Sex CA (Sex Chromosome Abnormalities), Tri: Trisomy

Complete trisomy 16 is the most frequent autosomal trisomy detected in spontaneous abortions in the first trimester due to incompatibility with life. On the other hand, partial trisomy of chromosome 16p has been reported in a few cases[31]. In one of our cases, partial trisomy 16 and partial monosomy 15 were observed as the result of unbalanced segregation of a maternally inherited balanced translocation: 46, XX, t(15;16) (q10;q11.2). In the majority of cases with partial trisomy 16, limb abnormalities, congenital heart defects, and facial features were reported to be consistent with our case[26]. Nevin et al.[32] presented a case suffering from dysmorphic facial features, patent ductus arteriosus, abnormal genitalia, and short survival with a der (15) t (15;16) (p12;q11) karyotype similar to our case's karyotype.

Reciprocal translocations and inversions are common chromosomal changes that are accepted as balanced at a microscopic level. On the other hand, approximately 6% of the balanced translocations and 9.4% of the balanced inversions have been reported to be associated with abnormal phenotypes[33]. We detected de novo balanced chromosomal rearrangements in five cases. These cases have a risk of submicroscopic deletions or duplications [33]. For further analysis, we recommended array comparative genomic hybridization testing at another genetic diagnosis laboratory.

Chromosomal mosaicism is one of the most challenging conditions in prenatal diagnosis due to the uncertainty of its effects on phenotype. Level III chromosomal mosaicism in amniocytes occurs in 0.1% - 0.3% of amniocentesis. Although Level II mosaicism is commonly pseudomosaicism, confirmational studies should not be ignored in the presence of abnormal USG findings[23]. Level II mosaicism was observed in five patients, and level III mosaicism was observed in one patient (0.1%). CS was recommended in these cases for confirmation.

The three most common indications were high serum screening tests, advanced maternal age, and abnormal USG findings; they were reported as 25%, 48%, and 11%, by Durmaz et al.[25] in Turkey; as 72%, 11%, and 14.5% by Younesi et al.[27]; as 44%, 30%, and 6% by Soler et al.[17]; and as 51%, 31%, and 26% by Andrew et al[24], each respectively. In our study, we detected these rates at 55%, 11%, and 29%, respectively. Abnormal USG findings tend to be higher than those of the studies by Durmaz et al.[25], Younesi et al.[27], Soler et al.[17] and Andrew et al.[24]

The age of 35 is considered an advanced maternal age in prenatal diagnosis and is associated with an increased risk of CAs[18]. In our patient group, CAs were found to be significantly higher ($p< 0.05$) in pregnant women above 35 years of age (independent of other indications) than in pregnant women below 35 years of age.

A combination of ultrasound markers and maternal serum markers in the first trimester of pregnancy can detect 97% of the fetuses with trisomy 21 and other major CAs. As they are screening tests, the results should be confirmed by a diagnostic test such as chromosome analysis, which is still accepted as the most appropriate method with a 99.4–99.8% diagnostic accuracy rate[34].

Of the patients who underwent amniocentesis with a high risk of screening tests, 94% had a high risk for trisomy 21 and 5.8% for trisomy 18. Trisomy 18 was observed in 10% of the patients with a high risk of trisomy 18. Trisomy 21 was observed in 2% of the patients with a high risk of trisomy 21. Structural chromosome abnormalities were observed in 3.5% of the patients with a high risk of trisomy 21. Cases with trisomy 5, trisomy 10, inv (16), del (16), and del13q were reported as level II mosaicism. CS and USG follow-up were recommended for these patients.

A second-trimester USG examination allows identification of women at risk for fetal aneuploidy. Sonographic markers are classified as major fetal structural abnormalities and soft markers. Soft markers are less substantial and may be temporarily seen in the normal fetus. The most frequently observed soft markers of aneuploidy are increased nuchal fold, single umbilical artery mild fetal pyelectasis, echogenic bowel, echogenic intracardiac focus, limb shortening, and choroid plexus cyst[34].

The most frequent soft marker was hyperechogenic bowel, which accounted for 10.5% (27/256) of all markers, followed by choroid plexus cyst 6.6% (17/256), pyelectasis 5% (13/256), echogenic intracardiac focus 3.5% (9/256), increased NT 3.5% (9/256), short tubular bones 2.3% (6/256), and single umbilical artery 1.5% (4/256). The chromosome abnormality rate was 14.6% among these groups.

Congenital heart diseases (CHDs) are frequently observed during prenatal diagnosis. Most types of CHDs can be cured with surgery and medical treatments after birth. CAs are observed in approximately 20% of the CHDs in prenatal diagnosis. The presence of chromosome

abnormalities in these cases may worsen the prognosis due to extracardiac anomalies such as neurodevelopmental disorders. Therefore, a prenatal genetic diagnosis should be recommended for fetuses with CHD[35].

In our group, the incidence of pathogenic CAs was 25% (16/62) of fetuses with sonographic cardiac defects. Additionally, 22q11 deletion syndrome (Di George syndrome) was tested by FISH in 5 cases with a sonographic cardiac defect, and all cases showed negative results.

Fetal central nervous system (CNS) anomalies are severe congenital anomalies and can be detected in the first and early second trimester. Genetic alterations are the primary cause of CNS anomalies. In this study, we recorded 85 extra or isolated CNS anomalies in 661 pregnant women. The most common anomalies were choroid plexus cysts, ventriculomegaly, and hydrocephalus[36]. CAs were detected in 11 cases with CNS anomaly (13%). Zhuang et al.[36] conducted karyotyping and chromosomal microarray analysis (CMA) on CAs associated with fetal CNS anomalies and revealed CAs at a rate of 4.35% with karyotyping and 22.3% with array CGH. A significant difference in the total CA detection rate with karyotyping (13% vs. 4.3%, $p < 0.001$) is comparable to the one in our study. On the other hand, the detection rate with array CGH was found to be high. It appears that array CGH is an appropriate approach for cases suffering from CNS anomalies with normal karyotypes.

Today, non-invasive prenatal diagnosis, based on cell-free fetal DNA analysis in maternal blood, is widely used to screen for fetal aneuploidies. Despite the high specificity and sensitivity capacity for trisomy 21 and trisomy 18, confirmation with a diagnostic test is still recommended for patients who have positive NIPT results[37].

In our group, one of the rare referral reasons for the invasive procedure was NIPT result confirmation with a 0.6% (n = 4) rate. Trisomy 21 was detected in three cases, and trisomy 18 in one. All these cases were confirmed in accordance with the NIPT results. We confirmed the cases with high-risk values, but the number of cases is insufficient to discuss the contribution of the test to prenatal diagnosis in our center.

Even though the results appear to be successful in evaluating the positive results by NIPT, the possibility of false negativity should not be ignored. In a study conducted in China, it was reported that non-invasive prenatal diagnosis missed the abnormal result confirmed by karyotyping at a rate of 12.4%[37].

There are some limitations to our study, including the lack of decisions and outcomes for pregnant women with abnormal karyotypes and a small sample size for indications with NIPT results.

This retrospective study presented the distribution of prenatal diagnosis indications and chromosome abnormalities in the region of Kocaeli. In our study, chromosomal anomalies with predicted risks (trisomies of 21, 18, and 13) constituted approximately half the abnormal karyotypes. Sex chromosome aneuploidies, rare aneuploidies, mosaicisms, and structural abnormalities involved the remaining part. This emphasizes that karyotyping provides comprehensive analysis regardless of the predicted risk or preliminary diagnosis. As a result, karyotyping plays an essential role in guiding genetic counseling for pregnant women at high risk for chromosomal diseases. In addition, the widespread use of genetic testing options can allow earlier diagnosis of fetal abnormalities. Prenatal diagnosis of genetic disorders is achieved more efficiently by combining genetic tests with different capacities.

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REFERENCES

1. Caceres V, Murray T, Myers C, Parbhoo K. Prenatal genetic testing and screening: a focused review. Semin Pediatr Neurol. 2022;42:100976.
2. Li H, Li Y, Zhao R, Zhang Y. Cytogenetic analysis of amniotic fluid cells in 4206 cases of high-risk pregnant women. Iran J Public Health. 2019;48:126–31.
3. Donze SH, Srebniak MI, Diderich KEM, van den Born M, Galjaard RJ, Govaerts LCP et al. Limited additional value of karyotyping cultured amniotic fluid cell colonies in addition to microarray on uncultured cells for confirmation of abnormal non-invasive prenatal testing results. Prenat Diagn. 2023; doi: 10.1002/pd.6499.
4. Manasatienkij C, Nimnual A, Jantarasaengaram S. Accuracy of prenatal diagnosis of common aneuploidies using QF-PCR comparing with standard

karyotyping in Thai pregnant women. Glob J Health Sci. 2022;31:341–52.
5. Carlson LM, Vora NL. Prenatal diagnosis: screening and diagnostic tools. Obstet Gynecol Clin North Am. 2017;44:245-56.
6. Grati FR. Chromosomal mosaicism in human feto-placental development: implications for prenatal diagnosis. J Clin Med. 2014;3:809-37.
7. Lim KMX, Mahyuddin AP, Gosavi AT, Choolani M. Genetics in prenatal diagnosis. Singapore Med J. 2023;64:27-36.
8. Zhang S, Yin M, Xu JZ, Lei CX, Wun JP, Sun XX et al. Cytogenetic analysis for fetal chromosomal abnormalities by amniocentesis : review of over 40,000 consecutive cases in a single center. Reprod Dev. 2017;1:84–8.
9. Shaffer LG, McGowan-Jordan J, Schmid M. International Standing Committee on Human Cytogenetic Nomenclature. ISCN: An International System for Human Cytogenetic Nomenclature. Basel, Karger, 2013.
10. Gardner RJ, Sutherland GR. Chromosome Abnormalities and Genetic Counselling. 233–246. New York, Oxford University Press, 2004.
11. Neagos D, Cretu R, Sfetea RC, Bohiltea LC. The importance of screening and prenatal diagnosis in the identification of the numerical chromosomal abnormalities. Maedica. 2011;6;179–84.
12. Türkyılmaz A, Budak T. Laboratuvarımıza prenatal tanı için sevk edilen ailelerde endikasyon ve sonuç uygunluklarının değerlendirilmesi. Dicle Tıp Dergisi. 2007;34:258–63.
13. Taşdemir Ş, Yılmaz M, Şahin İ, Erdem HB, Al RA, İngeç M et al. Retrospective analysis of 1429 cases who underwent amniocentesis and cordocentesis. Perinatal Journal. 2014;22:138-41.
14. Sala E, Conconi D, Crosti F, Villa N, Redaelli S, Roversi G. Interphase FISH: A helpful assay in prenatal cytogenetics diagnosis. OBM Genetics. 2019;3:1-12.
15. Simsek S, Turkyilmaz A, Oral D, Yalınkaya A, Tekes S, Isı H. The outcomes of prenatal karyotype analysis in amniocentesis and fetal blood sampling. International Archives of Medical Research. 2011;2:57–9.
16. An N, Li LL, Wang RX, Li LL, Yue JM, Liu RZ. Clinical and cytogenetic results of a series of amniocentesis cases from Northeast China : a report of 2500 cases. Genet Mol Res. 2015;14:15660–7.
17. Soler-Mademont I, Morales C, Clusellas N, Soler A, Sanchez A. Prenatal cytogenetic diagnosis in Spain : analysis and evaluation of the results obtained from amniotic fluid samples during the last decade. Eur J Obstet Gynecol Reprod Biol. 2011;157:156–60.
18. Xiao H, Yang YL, Zhang CY, Liao EJ, Zhao HR, Liao SX. Karyotype analysis with amniotic fluid in 12365 pregnant women with indications for genetic amniocentesis and strategies of prenatal diagnosis. J Obstet Gynaecol. 2016;36:293–6.
19. Sheth F, Rahman M, Liehr T, Desai M, Patel B, Modi C. Prenatal screening of cytogenetic anomalies – a Western Indian experience. BMC Pregnancy Childbirth. 2015;15:90.
20. Nishiyama M, Yan J, Yotsumoto J, Sawai H, Sekizawa A, Kamei Y et al. Chromosome abnormalities diagnosed in utero : a Japanese study of 28 983 amniotic fluid specimens collected before 22 weeks gestations. J Hum Genet. 2015;60:133-7.
21. Acar A, Ercan F, Yildirim S, Gorkemli H, Gezginc K, Balci O et al. Genetic amniocentesis results : analysis of the 3721 cases. The Medical Bulletin of Sisli Etfal Hospital. 2016;50:33–8.
22. Tao H, Xiao J, Yang C, Wang J, Tang Y, Guo C et al. Retrospective analysis of 4761 cases who underwent amniocentesis in southeast China. J Obstet Gynaecol. 2018;38:38–41.
23. Zhang S, Lei C, Wu J, Sun H, Yang Y, Zhang Y et al. A retrospective study of cytogenetic results from amniotic fluid in 5328 fetuses with abnormal obstetric sonographic findings. J Med Ultrasound. 2017;36:1809–17.
24. Andrew C, Koshy T, Gopal S, Paul SFD. A retrospective exploratory study of fetal genetic invasive procedures at a University Hospital. J Obstet Gynaecol. 2018;38:906–10.
25. Durmaz B, Bolat H, Cengisiz Z, Akercan F, Sözen Türk T, Parıltay E et al. 20-year experience on prenatal diagnosis in a reference university medical genetics center in Turkey. Turk J Med Sci. 2021;51:1775-80.
26. Bozdoğan Tuğ S, Büyükkurt S, Özer S, Bişgin A. Evaluation of the results of patients who applied to the Çukurova University, Medical Genetics Department for prenatal diagnosis and determination of genetic counseling principles. Turk J Med Sci. 2021;51:657-60.
27. Younesi S, Taheri Amin MM, Hantoushzadeh S, Saadati P, Jamali S, Modarressi MH et al. Karyotype analysis of amniotic fluid cells and report of chromosomal abnormalities in 15,401 cases of Iranian women. Sci Rep. 2021;11:19402.
28. López Rivera JJ, Zapata Arizabaleta M, Castañeda Soler DP, Carrillo YD, Gualdron López O, Forero-Castro M. Prenatal cytogenetic diagnosis: results obtained in the specialized laboratory of Clínica Universitaria Colombia from 2013 to 2019. J Matern Fetal Neonatal Med. 2022;35:7430-7.
29. Moczulska H, Chrzanowska-Steglinska M, Skoczylas B, Wojda K, Borowiec M, Sieroszewski P. Prenatal karyotype results from 2169 invasive tests. Ginekol Pol. 2023; doi:10.5603/GP.a2022.0143.
30. Wang T, Ren C, Chen D, Lu J, Guo L, Zheng L et al. Prenatal diagnosis of Pallister-Killian syndrome using cord blood samples. Mol Cytogenet. 2019;12:39.
31. Mishra R, Paththinige CS, Sirisena ND, Nanayakkara S, Kariyawasam UGIU, Dissanayake VHW. Partial

trisomy 16q21 ➔ qter due to an unbalanced segregation of a maternally inherited balanced translocation review of literature. BMC Pediatr. 2018;18:4.
32. Nevin NC, Coffey WW, Nevin J, Reid M. Partial trisomy 16q in two boys resulting from a maternal translocation, t(15;16)(p12; q11). Clin Genet. 1983;24:375–9.
33. Simioni M, Artiguenave F, Meyer V, Sgardioli IC, Viguetti-Campos NL, Lopes Monlleó I et al. Genomic investigation of balanced chromosomal rearrangements in patients with abnormal phenotypes. Mol Syndromol. 2017;8:187-94.
34. Ali MK, Shazly SA, Ali AH, Abdelbadee AY, Abbas AM. Ultrasonographic soft markers of aneuploidy in second trimester fetuses. Middle East Fertil Soc J. 2012;17:145–51.
35. Wang Y, Cao L, Liang D, Meng L, Wu Y, Qiao F et al. Prenatal chromosomal microarray analysis in fetuses with congenital heart disease: a prospective cohort study. Am J Obstet Gynecol. 2018;218:244.e1-17.
36. Zhuang J, Zhang N, Chen Y, Jiang Y, Chen X, Chen W et al. Prenatal diagnosis and molecular cytogenetic characterization of fetuses with central nervous system anomalies using chromosomal microarray analysis: a seven-year single-center retrospective study. Sci Rep. 2024;14:2271.
37. Chen Y, He Z, Shi Y, Zhou Q, Cai Z, Yu B et al. Not all chromosome aberrations can be detected by NIPT in women at advanced maternal age: A multicenter retrospective study. Clin Chim Acta. 2018;486:232–6.