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## Indications and results of prenatal invasive diagnosis (amniocentesis, chorionic villus sampling, cordocentesis): A tertiary center experience

### *Prenatal invaziv tanı endikasyonları ve sonuçları (amniyosentez, koryon villus örnekleme, kordosentez): Tersiyer bir merkez deneyimi*

Zeynep Seyhanli \*<sup>1</sup> , Betül Tokgoz Cakir <sup>1</sup> , Can Ozan Ulusoy <sup>1</sup> , Sadun Sucu <sup>1</sup> , Gulsan Karabay <sup>1</sup> , Abdüllatif Bakır <sup>2</sup> , Can Tekin Iskender <sup>1</sup> , Şevki Çelen <sup>1</sup> 

<sup>1</sup> Ankara Etlik City Hospital, Department of Perinatology, Ankara, Türkiye

<sup>2</sup> Ankara Etlik City Hospital, Department of Medical Genetics, Ankara, Türkiye

#### Abstract

**Objective:** To evaluate the indications for prenatal invasive procedures and karyotype results in pregnant women

**Materials and Methods:** We conducted a retrospective analysis of the indications and karyotype results of pregnant women who opted for invasive diagnostic testing in a tertiary center between September 2022 and May 2023.

**Results:** Of the 331 patients who underwent prenatal genetic diagnosis, 267 underwent amniocentesis (80.7%), 62 underwent chorionic villus sampling (18.7%), and 2 underwent cordocentesis (0.6%). The most common indication for amniocentesis was an elevated risk in first-trimester screening tests (29.6%). Chorionic villus sampling was most frequently performed due to an increased risk in the first-trimester screening test (37.1%) and because of an increased nuchal translucency (37.1%). Cordocentesis was exclusively performed in cases of abnormal second-trimester ultrasound examinations. Trisomy 21 was most frequently detected in cases with increased nuchal translucency (13.3%).

**Conclusions:** The importance of invasive prenatal diagnostic procedures for the early detection and diagnosis of genetic disorders and the assessment of fetal health is evident. However, the decision to undergo these procedures should be made thoughtfully, with careful consideration of patient counselling and informed consent.

**Keywords:** amniocentesis; chorionic villus sampling; cordocentesis

## Öz

**Amaç:** Gebelerin prenatal invaziv işlem endikasyonlarını ve karyotip sonuçlarını değerlendirmek.

**Gereç ve Yöntem:** Eylül 2022 ile Mayıs 2023 tarihleri arasında tersiyer bir merkezde invaziv tanı testi önerilen gebe kadınlar için endikasyonların ve karyotip sonuçlarının retrospektif bir analizini yaptık.

**Bulgular:** Prenatal genetik tanı konulan 331 hastanın 267'sine amniyosentez (%80.7), 62'sine koryon villus örnekleme (%18.7) ve 2'sine (%0.6) kordosentez uygulandı. Amniyosentez için en sık endikasyon ilk trimester tarama testlerinde riskin yüksek çıkmasıydı (%29.6). İlk trimester tarama testinde riskin artışı (%37.1) ve ense kalınlığının artışı (%37.1) nedeniyle en sık koryon villus örnekleme yapıldı. Kordosentez yalnızca anormal ikinci trimester ultrason muayenesi vakalarında yapıldı. Trizomi 21 en sık ense kalınlığının arttığı olgularda (%13.3) tespit edildi.

**Sonuç:** Genetik bozuklukların erken tespiti ve tanısı ile fetal sağlığın değerlendirilmesinde invaziv prenatal tanı prosedürlerinin önemi açıktır. Ancak bu prosedürleri uygulama kararı, hasta danışmanlığı ve bilgilendirilmiş onam dikkate alınarak dikkatli bir şekilde verilmelidir.

**Anahtar Kelimeler:** amniyosentez; koryon villus örnekleme; kordosentez

## 1. Introduction

Approximately 3–5% of pregnancies are complicated by congenital diseases or genetic disorders (1). Ultrasound and maternal serum markers are commonly utilized for screening these conditions during the prenatal period. If there is a clinical suspicion, prenatal genetic diagnostic tests are essential to determine the genetic background and diagnose existing diseases. Invasive prenatal diagnosis is the procedure for obtaining fetal or embryo-fetal tissue that is useful for the diagnosis of chromosomal and/or genetic pathologies (2). Prenatal genetic testing is performed by genetic analysis of samples obtained by chorionic villus sampling, amniocentesis, and cordocentesis. CVS is performed between 10 and 14 weeks' gestation in the first trimester, amniocentesis may be performed at any gestational age after 15 weeks and cordocentesis is obtained from the umbilical vein usually at 18-23 weeks of gestation under ultrasound guidance (3). Indications for these tests include increased nuchal translucency, abnormal ultrasound findings in the first and second trimester, an elevated risk in screening tests, a family history of genetic abnormalities, and the possibility of fetal transmission of maternal infections (4). The results form the basis for medical decisions. Early detection of genetic problems plays a crucial role in initiating appropriate treatment and referral. However, prenatal genetic testing carries potential risks for maternal and fetal complications. This article looks at the medical practice and outcomes of prenatal genetic diagnostics, shedding light on this critical issue through the experience of a single tertiary center. We will also examine in detail the benefits of prenatal genetic diagnostics for healthcare professionals and expectant mothers, as well as the potential risks associated with them.

## 2. Materials and methods

This retrospective analysis involved the examination of the records of all patients who underwent prenatal genetic diagnosis at Ankara Etlik City Hospital Perinatology Clinic between September 2022 and May 2023. The study was ethically approved at the beginning (Decision Date No.: AEŞH-EK1-2023-351). The data were collected in the hospital's electronic database.

Invasive genetic screening has been recommended for the following indications:

- Increased nuchal translucency (NT) ( $\geq 3$  mm)
- Abnormal ultrasound findings in the first trimester (e.g., megacystis, omphalocele, when NT  $< 3$  mm)
- Abnormal ultrasound findings in the second trimester
- Increased risk in double screening tests for Trisomy 21 (Trisomy 21  $> 1/270$ ) or Trisomy 13/18 (Trisomy 13/18  $> 1/150$ )
- Increased risk in triple screening tests for Trisomy 21 (Trisomy 21  $> 1/270$ ) or Trisomy 18 (Trisomy 18  $> 1/150$ )
- Abnormal result on non-invasive prenatal test (NIPT)
- A family history of genetic abnormalities
- Maternal CMV or Toxoplasma IgM positivity
- Advanced maternal age ( $\geq 35$  years with negative serum screening and normal ultrasound findings)
- Unsuccessful chorionic villus sampling
- Family request

Before each prenatal invasive procedure, all patients were informed thoroughly and in detail about the procedure and

the potential complications that could arise during or after the process. Parental consent was obtained before proceeding with the prenatal diagnosis. Subsequently, all biological materials collected during the procedures were dispatched to the same genetic laboratory for comprehensive genetic analysis. Prophylactic antibiotics were administered to all patients prior to the invasive procedure to minimise the risk of infection. In cases where Rh incompatibility was identified, Rh immune globulin G (Rh IgG) was administered to patients.

### Statistical Analysis

Statistical Package for the Social Sciences (SPSS) v26.0 (IBM® SPSS® Statistics, New York, USA) was used for the statistical analysis. Data were retrieved from the hospital's electronic database. Counts and percentages were chosen as the primary metrics for the data presentation.

### 3. Results

Of the 331 patients who underwent prenatal genetic diagnosis, 267 (80.7%) underwent amniocentesis, 62 (18.7%) underwent chorionic villus sampling, and 2 (0.6%) underwent cordocentesis. The average age of the pregnant women participating in the study was 31.4±6.7. The average body mass index (BMI) was 26.4±3.4, and 231 (71%) of the patients were observed to be multiparous. The most common indication for amniocentesis was an increased risk for a first-trimester screening test (29.6%). Other reasons for amniocentesis, in descending order of frequency, were abnormal ultrasound

findings in the second trimester (27.7%), an increased risk due to a triple screening test (24.3%), a pregnancy history involving genetic abnormalities (4.9%), increased nuchal translucency (2.6%), advanced maternal age (2.6%), an abnormal NIPT result (2.2%), failed chorionic villus sampling (1.9%), Toxoplasma IgM positive (1.5%), CMV IgM positive (1.5%), family request (0.7%), and an abnormal first-trimester ultrasound (0.4%). Cordocentesis was only performed for abnormal ultrasound examinations in the second-trimester. Chorionic villus sampling was most frequently performed because of an increased risk in the first trimester screening test (37.1%) and because of an increased nuchal translucency (37.1%). Other indications for CVS were abnormal ultrasound examinations in the second trimester (9.7%), increased maternal age risk (6.5%), pregnancy with a history of genetic abnormalities (6.5%), and abnormal ultrasound examinations in the first trimester (3.2%) (Table 1).

When considering all indications for genetic analysis, the most common result was a normal karyotype. Normal karyotypes were found in all analyses conducted for indications such as advanced maternal age, CMV-IgM positivity, unsuccessful chorionic villus sampling, and familial enquiries. In the analysis performed with the indication of increased risk of the triple screening test, a structural abnormality was detected in only 2 patients (3.1%). The problem of production of genetic material production in the culture medium was most frequently observed in cases related to a pregnancy history with genetic abnormalities (5.9%). Trisomy 21 was most frequently detected

n,%	Chorionic Villus Sampling	Amniocentesis	Cordocentesis
Increased risk of double screening test	23 (37.1%)	79 (29.6%)	0
NT increase	23 (37.1%)	7 (2.6%)	0
Increased risk of triple screening test	0	65 (24.3%)	0
Second trimester abnormal ultrasound	6 (9.7%)	74 (27.7%)	2 (%100)
Unsuccessful chorionic villus sampling	0	5 (1.9%)	0
First trimester abnormal ultrasound	2 (3.2%)	1 (0.4%)	0
CMV IgM positivity	0	4 (1.5%)	0
Abnormal result on NIPT	0	6 (2.2%)	0
Pregnancy history with genetic anomalies	4 (6.5%)	13 (4.9%)	0
Toxoplasma IgM positivity	0	4 (1.5%)	0
Maternal age risk	4 (6.5%)	7 (2.6%)	0
Family request	0	2 (0.7%)	0
Total	62 (%100)	267 (%100)	2 (%100)

Abbreviations: NT: Nuchal translucency, CMV: Cytomegalovirus, NIPT: Non invasive prenatal test



**Table 2. Fetal karyotype results according to indications**

n, %	45,X0	47,XXY	47,XXX	Structural abnormality	Maternal contamination	Normal	Trisomy 18	Trisomy 21	No growth in culture	Total
Increased risk of double screening test	1 (1%)	2 (2%)	0	1 (1%)	0	95 (93.1%)	0	0	3 (2.9%)	102 (100%)
NT increase	1 (3.3%)	0	0	0	0	23 (76.7%)	1 (3.3%)	4 (13.3%)	1 (3.3%)	30 (100%)
Increased risk of triple screening test	0	0	0	2 (3.1%)	0	63 (96.9%)	0	0	0	65 (100%)
Second trimester abnormal ultrasound	0	0	0	4 (4.9%)	3 (3.7%)	70 (85.4%)	1 (1.2%)	3 (3.7%)	1 (1.2%)	82 (100%)
Unsuccessful chorionic villus sampling	0	0	0	0	0	5 (100%)	0	0	0	5 (100%)
First trimester abnormal ultrasound	0	0	0	0	1 (33.3%)	2 (66.7%)	0	0	0	3 (100%)
CMV IgM positivity	0	0	0	0	0	4 (100%)	0	0	0	4 (100%)
Abnormal result on NIPT	1 (16.7%)	0	1 (16.7%)	0	0	4 (66.7%)	0	0	0	6 (100%)
Pregnancy history with genetic anomalies	0	0	0	3 (17.6%)	0	13 (76.5%)	0	0	1 (5.9%)	17 (100%)
Toxoplasma IgM positivity	0	0	0	0	0	4 (100%)	0	0	0	4 (100%)
Maternal age risk	0	0	0	0	0	11 (100%)	0	0	0	11 (100%)
Family request	0	0	0	0	0	2 (100%)	0	0	0	2 (100%)

Abbreviations: NT: Nuchal translucency, CMV: Cytomegalovirus, NIPT: Non invasive prenatal test

in cases with increased nuchal translucency (13.3%). Two patients with Trisomy 18 had evidence of abnormal second-trimester ultrasound and increased nuchal translucency. Of the abnormal results with the NIPT indication, 66.7% had a normal karyotype, 1 patient had 45,X0, and 1 patient had 47,XXY. The distribution of genetic analysis results based on interventional procedure indications is outlined in Table 2.

Patients aged 35 years and older and those younger than 35 years were most likely to have a normal karyotype (89.4%). Trisomy 21, Trisomy 18, maternal contamination, and 47,XXY were more common in patients aged 35 years and older, while 45,X0, structural abnormalities, and genetic culture defects were more common in patients younger than 35 years (Table 3).

#### 4. Discussion

In this study, it was demonstrated that the most common prenatal invasive test was performed due to an increased risk revealed by the double screening test. Additionally, the study revealed that the most frequent pathological findings were Trisomy 21, one of the aneuploidies, and structural anomalies, which belong to the category of other anomalies. We found abnormal genetic results in 6.04% of high-risk pregnancies that were referred to our perinatology department, a result that aligns with existing literature. Despite the study's limited sample size, this rate is consistent with estimates found in the literature.

Chromosomal abnormalities have been reported to occur in approximately 1 in 150 live births (5). However, because aneuploidies are responsible for most early pregnancy losses, the prevalence of chromosomal abnormalities is higher in the

early stages of pregnancy. Factors associated with an increased likelihood of a chromosomal abnormality include advancing maternal age, a parental history of genetic abnormalities, a previous pregnancy with a chromosomal abnormality, prenatal ultrasound abnormalities, or a positive screening test result (6). In our study, the most common indication for amniocentesis was an elevated risk identified during the first-trimester screening test (29.6%), followed by abnormal second-trimester ultrasound findings (27.7%).

Maternal serum markers and ultrasound screening methods are employed to identify high-risk pregnancies related to chromosomal abnormalities. In cases with high risk, invasive diagnostic tests such as amniocentesis, chorionic villus sampling, and cordocentesis become necessary (3). We performed prenatal genetic diagnosis by amniocentesis in 267 (80.7%), chorionic villus sampling in 62 (18.7%), and cordocentesis in 2 (0.6%) of the 331 patients.

Options for prenatal genetic screening (serum screening with or without nuchal translucency ultrasound or cell-free DNA screening) and diagnostic testing (CVS, amniocentesis or cordocentesis) should be discussed and offered to all pregnant patients, regardless of age or risk for chromosomal abnormalities (6).

First-trimester screening offers the advantage of earlier diagnosis and can also screen for other structural, genetic, or placental disorders, as well as additional aneuploidies (7). The triple screening test, while providing a lower sensitivity for the detection of Trisomy 21 (with a sensitivity of 69% and a 5% positive screening test result rate), is less effective than first-trimester screening (8). We did not find Trisomy 13,18 or 21 results in any of our patients who were classified as high-risk during double and triple screening tests who underwent prenatal invasive diagnostic test. However, in the cases where invasive procedures were performed with the indication of an isolated NT  $\geq 3$ mm, we referred them directly to the invasive procedure, without performing double and triple screening tests, in accordance with the literature. This may have resulted in the trisomy cases we detected being diagnosed prior to the screening tests. We detected a 45,X0 chromosomal abnormality in one case and a 47,XXY chromosomal abnormality in two cases who underwent prenatal invasive diagnostic testing due to the high values of the double screening test.

An increased NT value elevates the risk of genetic syndromes and anomalies, even if the chromosomes appear normal on diagnostic tests (9). NT is the primary sonographic marker in the first trimester, and when used alone to modify the age-related risk of Trisomy 21, the detection rate is approximately

n,%	<35 years	$\geq 35$ years
45,X0	3 (1.4%)	0
47,XXY	0	2 (1.6%)
47,XXX	1 (0.5%)	0
Structural abnormality	8 (3.8%)	2 (1.6%)
Maternal contamination	2 (1%)	2 (1.6%)
Normal	186 (89.4%)	110 (89.4%)
Trisomy 18	1 (0.5%)	1 (0.8%)
Trisomy 21	3 (1.4%)	4 (3.3%)
No growth in culture	4 (1.9%)	2 (1.6%)
Total	208 (%100)	123 (%100)

70% (10). In our study, 30 cases exhibited an increase in NT, and 6 (20%) of them were found to have chromosomal anomalies. This underscores the significance of NT measurements during first-trimester ultrasound examinations.

Regardless of screening or diagnostic testing, all patients should be offered a second-trimester ultrasound scan to detect structural abnormalities (11). Only about 27% of fetuses diagnosed with Trisomy 21 have a major structural abnormality on ultrasound in the second trimester (12). In our cases, the anomaly was detected on second trimester ultrasound in 3 of the 7 cases in which Trisomy 21 was detected (42.8%).

NIPT as a complementary or alternative method is the most sensitive screening option for Trisomy 21, 18 and 13, the aneuploidies that account for 71 percent of all prenatally detected chromosomal abnormalities (13). However, it's important to note that NIPT is not considered a diagnostic test, as false-positive and false-negative results can occur (6). Invasive procedures, in conjunction with fetal ultrasound and microarray testing, enable the detection of many additional congenital abnormalities not yet identified by NIPT (14). In our study, the genetic results of 6 patients with abnormal NIPT test results after invasive testing resulted as 45,X0 in one, 47,XXX in one and a normal karyotype in 4 of them and these findings supported that NIPT is not a diagnostic test.

The strengths of this study include the detailed description of the specific indications and genetic results of the invasive testing in the entire patient population, as well as the inclusion of comprehensive physician data. The study's weaknesses lie in its relatively small cohort over a 9-month period and the reliance on an electronic database for all data due to its retrospective design.

Prenatal invasive testing for chromosomal abnormalities enables the accurate determination of a patient's risk of carrying a fetus with such abnormalities. The results presented in this study reiterate the significance of invasive prenatal diagnostic procedures in obstetrics. These techniques continue to be indispensable for the early detection and diagnosis of genetic disorders and for assessing fetal health. Nonetheless, the decision to undergo these procedures should be made thoughtfully, with due consideration for patient counseling and informed consent.

#### Author contribution

Study conception and design: ZŞ and BTÇ; data collection: COU, CTİ and AB; analysis and interpretation of results: ZŞ, SS, GK and ŞÇ; draft manuscript preparation: ZŞ, BTÇ, AB and CTİ. All authors reviewed the results and approved the final version of the manuscript.

#### Ethical approval

The study was approved by the Ethics Committee for Noninterventional Studies of Ankara Etlik City Hospital (Decision no: AEŞH-EK1-2023-351).

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#### Conflict of interest

The authors declare that there is no conflict of interest.

#### Yazar katkısı

Araştırma fikri ve tasarımı: ZŞ ve BTÇ ; veri toplama: COU, CTİ ve AB ; sonuçların analizi ve yorumlanması: ZŞ, SS, GK ve ŞÇ ; araştırma metnini hazırlama: ZŞ, BTÇ, AB ve CTİ. Tüm yazarlar araştırma sonuçlarını gözden geçirdi ve araştırmanın son halini onayladı.

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