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Karyotype analysis of fetus in pregnant women with different indications for amniocentesis.

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Keywords: prenatal diagnosis; amniocentesis; karyotype analysis; chromosomal abnormalities.

Abstract. To analyze the karyotype distribution in 1285 pregnant women and evaluate the association between karyotype and diagnostic indications of fetal chromosomal abnormalities, 1285 pregnant women with prenatal diagnostic indications and successful amniocentesis admitted to our hospital from July 2019 to June 2022 were selected as study subjects for fetal karyotype analysis. The distribution of prenatal diagnostic indications and abnormal karyotypes were recorded, and the association between abnormal karyotypes and different diagnostic indications was analyzed. Ninety-six abnormal chromosomal karyotypes in amniotic fluid cells were detected in the samples, with an abnormality rate of 7.47%. Chromosome numerical abnormalities accounted for 70.83% (68/96), and the detection rate was 5.29% (68/1285), the most common category of abnormal kariotypes, trisomy 21, was the most common among them, accounting for 44.79% (43/96). Advanced maternal age and high risk of serologic screening were the main indications for prenatal diagnosis. The highest detection rates were for abnormal non-invasive prenatal DNA testing and one parent carrying chromosome abnormality, 27.63% and 42.86%, respectively. Karyotype analysis of pregnant women with indications for amniocentesis is effective in screening for fetal chromosomal abnormalities and reducing congenital anomalies.

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Análisis del cariotipo fetal de embarazadas con diferentes indicaciones de amniocentesis.

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Palabras clave: diagnóstico prenatal; amniocentesis; análisis del cariotipo; anomalías cromosómicas.

Resumen. El objetivo fue analizar la distribución del cariotipo de mujeres embarazadas y evaluar la asociación entre el cariotipo y las indicaciones diagnósticas de anomalías cromosómicas fetales. Un total de 1285 mujeres embarazadas, con indicaciones de diagnóstico prenatal y amniocentesis exitosa, admitidas en nuestro hospital desde julio de 2019 hasta junio de 2022 fueron seleccionadas como sujetos de estudio para el análisis del cariotipo fetal. Se registró la distribución de las indicaciones de diagnóstico prenatal y los cariotipos anormales, y se analizó la asociación entre los cariotipos anormales y las diferentes indicaciones de diagnóstico. En las muestras se detectó un total de 96 cariotipos cromosómicos anómalos en células de líquido amniótico, con una tasa de anormalidad del 7,47%. Las anomalías numéricas cromosómicas representaron el 70,83% (68/96) y la tasa de detección fue del 5,29% (68/1285), siendo la categoría más común de cariotipos anormales. Entre ellas, la trisomía 21 fue la más frecuente, con un 44,79% (43/96). La edad materna avanzada y el alto riesgo de cribado serológico fueron las principales indicaciones para el diagnóstico prenatal. Las tasas de detección más elevadas correspondieron a las pruebas prenatales no invasivas de ADN anómalo y a un progenitor portador de anomalía cromosómica, 27,63% y 42,86%, respectivamente. En conclusión, el cariotipo de las mujeres embarazadas con indicación de amniocentesis es un cribado eficaz para detectar anomalías cromosómicas fetales y reducir las anomalías congénitas.

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INTRODUCTION

Chromosomal abnormalities are a common clinical genetic disorder that affects one in every 150 infants [1]. Infants with these disorders are often mentally disabled, with a variety of malformations and stunting [2]. At present, there is no effective treatment for this type of disease. The primary reliance is on prenatal screening and termination of pregnancy to avoid the birth of such fetuses. The standard prenatal diagnostic methods are divided into two categories: non-invasive prenatal testing and invasive prenatal testing[3]. Non-invasive prenatal DNA testing (NIPT), ultrasonography and maternal serum screening are commonly used non-invasive screening methods [2, 4, 5]. However, these methods cannot accurately detect genes in fetal cells, and there are cases of missed diagnosis and misdiagnosis [6]. Karyotyping after amniocentesis is still the gold standard for detecting fetal chromosomal abnormalities [7].

Amniocentesis is usually performed at 18-24 weeks of pregnancy to detect fetal chromosomal conditions by extracting and culturing amniotic fluid cells [8]. Karyotype analysis is highly specific and sensitive, and the diagnostic rate of fetal chromosomal ab-

normalities is almost 100% [9]. Prenatal diagnostic indications for amniocentesis usually include abnormal NIPT, high-risk maternal serum screening, advanced maternal age, abnormal ultrasonographic indications, paternal or maternal carrying chromosome abnormalities, and adverse pregnancy history [10, 11].

Despite the wealth of data available on the outcomes of amniocentesis, gaps remain in the literature, particularly concerning the comprehensive analysis of karyotype results by different indications for the procedure. Previous studies have often focused on single indications or a small subset of chromosomal abnormalities. The novelty of the present study lies in its comprehensive approach, analyzing a large, diverse cohort with a wide range of indications for amniocentesis. Therefore, this study aimed to assess the relationship between various prenatal diagnostic indications and fetal chromosomal abnormalities by performing a karyotype analysis of amniotic fluid cells in 1285 instances by amniocentesis in high-risk pregnant women.

PATIENTS AND METHODS

Sample collection

Our study design type is a present retrospective study. One thousand two hundred eighty-five pregnant women admitted to our hospital for amniotic fluid karyotyping from July 2019 to June 2022 were selected as the study population. The pregnant women aged 19 to 46 and 18 to 24 weeks of gestation. Detailed demographic and clinical data were gathered by completing questionnaires, conducting direct interviews with pregnant women, and assessing medical records. The study was approved by the First Affiliated Hospital ethics committee of Soochow University, and all participants were informed and signed the consent form.

Inclusion criteria: presence of prenatal diagnostic indications, including high risk of NIPT, advanced maternal age, high risk of serologic screening, abnormal ultrasonographic indications, history of adverse pregnancy and one parent carrying chromosomal abnormalities; amniocentesis for the first time.

Exclusion criteria: unsuccessful cultures of amniotic fluid cells twice; the presence of threatened abortion.

Amniocentesis

The pregnant women and their families were informed of the risks, and an amniocentesis was performed after signing the informed consent. Twenty mL of amniotic fluid was drawn from the pregnant women and centrifuged at 2000 r/min for 10 minutes. After centrifugation, the supernatant was discarded, and the cell suspension was inoculated in the amniocyte culture medium at 37°C and 5% CO_2 for 9 to 10 days. The amniotic fluid cells were collected when multiple clones with various metaphase cells were observed with an inverted microscope. After harvesting the cells, g-band staining was performed and canned by a Leica GLS120 Automated Nuclear Scanning System. Thirty karyotypes were routinely counted, and five karyotypes were analyzed following the International System for Human Cytogenetic Nomenclature (ISCN) standard [12].

Statistical analysis

Statistical analysis was performed using the IBM® SPSS 24.0® software. Count data were expressed using frequency and rate (%) and analyzed by chi-square test. $p<0.05$ was considered a statistically significant difference.

RESULTS

Distribution of different diagnostic indications

Among the 1285 pregnant women with diagnostic indications, most cases were prompted by high-risk maternal serum screening, accounting for 573 cases (44.59%), followed by advanced maternal age with 522 cases (40.62%). Less frequent indications included abnormal results from non-invasive prenatal testing (NIPT), which

led to 76 cases (5.91%), and abnormal ultrasonographic findings, which accounted for 67 cases (5.21%). The least common reasons for amniocentesis were a history of adverse pregnancy outcomes and parental chromosomal abnormalities, with 26 cases (2.02%) and 21 cases (1.63%) respectively (Table 1).

Table 1
Distribution of different diagnostic indications.

Clinical indicator	Cases	Proportion (%)
High-risk maternal serum screening	573	44.59
Advanced maternal age	522	40.62
Abnormal ultrasonographic indications	67	5.21
Abnormal NIPT	76	5.91
Paternal/maternal carrying chromosome abnormality	21	1.63
Adverse pregnancy history	26	2.02
Total	1285	100

Classification and detection rate of abnormal karyotypes

The study examined 1,285 cases of amniotic fluid samples from high-risk pregnant women. Of these, 96 (7.47%) cases showed chromosomal abnormalities. Numerical abnormalities were the most common, accounting for 68 (70.83%) abnormal karyotypes. The most frequent numerical abnormality was Trisomy 21, which was observed in 43 (44.79%) cases, followed by Trisomy 18 in 10 (10.42%) cases, Trisomy 13 in 2 (2.08%) cases, 47 XXX (trisomy X) in 2 (2.08%) cases, 47 XYY (Jacob's syndrome) in 1 (1.04%) case, and 47 XXY (Klinefelter syndrome) in 2 (2.08%) cases. Additionally, 1 (1.04%) case of 45 X (Turner Syndrome or TS) was detected. Structural abnormalities were observed in 28 (29.17%) cases, including 6 (6.25%) translocations, 13 (13.54%) inversions, and 9 (9.38%) chromosome polymorphisms (Table 2).

Table 2
Classification and detection rate of abnormal karyotypes.

Chromosomal karyotype	Number (n)	Occupancy% (n/96)	Detection rate% (n/1285)
Numerical abnormalities	68	70.83	5.29
Trisomy 21	43	44.79	3.35
Trisomy 18	10	10.42	0.78
Trisomy 13	2	2.08	0.16
47, XXX	2	2.08	0.16
47, XYY	1	1.04	0.08
47, XXY	2	2.08	0.16
45, X	1	1.04	0.08
Mosaicism	7	7.29	0.54
Structural abnormalities	28	29.17	2.18
Translocation	6	6.25	0.47
Inversion	13	13.54	1.01
Chromosome polymorphism	9	9.38	0.70
Total	96	100.00	7.47

Distribution of diagnostic indications in pregnant women with amniotic fluid karyotype abnormalities

The abnormal karyotype detection rate of one parent carrying a chromosome abnormality was 42.86% (9/21; calculation formula of detection rate (%): n/number of pregnant women per diagnostic indication in 1285 cases (Table 3)), which was the highest detection rate of all indications, followed by high-risk NIPT (27.63%) and abnormal ultrasonographic indications (14.93%). The more significant number of karyotype abnormalities was found in advanced maternal age, accounting for 30.21% (29/96), followed by high-risk maternal serum (26.04%) and abnormal NIPT (21.88%).

Distribution of abnormal karyotypes for different prenatal diagnostic indications

As shown in Table 4, trisomy 21 was most common in pregnant women with advanced maternal age. Trisomy 21 also has the highest proportion in other abnormal karyotypes of older pregnant women. Fetal chromosomal structural abnormalities are mainly distributed in abnormal serological screening, and one parent carries the abnormal chromosome. Fetal chromosomal numerical abnormalities are mainly distributed in advanced maternal age, abnormal NIPT, and high-risk maternal serum screening. However, in terms of the total number, fetal chromosomal abnormalities are mainly distributed between advanced maternal age and high-risk maternal serum screening.

DISCUSSION

This study aimed to assess the relationship between various prenatal diagnostic indications and fetal chromosomal abnormalities by performing a karyotype analysis of amniotic fluid cells in 1,285 high-risk pregnant women. The most common indications for amniocentesis were high-risk maternal serum screening (44.59%) and advanced maternal age (40.62%), followed by abnormal NIPT results (5.91%) and abnormal ultrasonographic findings (5.21%). Karyotype analysis detected chromosomal abnormalities in 96 cases, resulting in an abnormality rate of 7.47%. Numerical chromosomal abnormalities were more common, accounting for 70.83% of the abnormalities, with trisomy 21 being the most frequent (44.79%). Structural chromosomal abnormalities comprised 29.17% of the abnormalities, with inversions being the most common (13.54%).

The findings of this study are consistent with previous research on the distribution of prenatal diagnostic indications and the detection rates of chromosomal abnormalities. A study by Grgić *et al*. reported that the most common indication for amniocentesis was advanced maternal age [13]. Additionally, a study by Golshahi *et al.* reported that the most common indication for amniocentesis

Table 3

Distribution of diagnostic indications in pregnant women with amniotic fluid karyotype abnormalities.

Clinical indication	Abnormal karyotype(n)	Detection rate (%)	Proportion (%)
High-risk maternal serum screening	25	4.36	26.04
Advanced maternal age	29	5.56	30.21
Abnormal ultrasonographic indications	10	14.93	10.42
Abnormal NIPT	21	27.63	21.88
Paternal/maternal carrying chromosome abnormality	9	42.86	9.38
Adverse pregnancy history	2	7.69	2.08
Total	96	7.47	100.00

Table 4
Distribution of abnormal karyotypes for different prenatal diagnostic indications.

Diagnosis indicator	Chromosomal karyotype						
	a	b	c	d	e	f	Total
Trisomy 21	7	23	5	8			43
Trisomy 18	2	3	2	3			10
Trisomy 13	1			1			2
47, XXX	1		1				2
47, XYY				1			1
47, XXY	2						2
45, X		1					1
Mosaicism	3			4			7
Translocation	1		2	2	1		6
Inversion	3	2		2	6		13
Chromosome polymorphism	5				2	2	9
Total	25	29	10	21	9	2	96

a, High-risk maternal serum screening; b, Advanced maternal age; c, Abnormal ultrasonographic indications; d, Abnormal NIPT; e, Paternal/maternal carrying chromosome abnormality; f, Adverse pregnancy history.

was abnormal serum screening, similar to the results of the current study [14]. Also, this study's overall chromosomal abnormality detection rate of 7.47% is similar to other studies [15, 16]. However, the study of Sun *et al*. reported the detection rate of abnormal karyotypes to be 2.02%, which may be due to the sample size and methods used [17].

The high detection rate of numerical chromosomal abnormalities, particularly trisomy 21, is well-documented in the literature. A review by Liu *et a*l. found that trisomy 21 was the most common chromosomal abnormality detected prenatally, accounting for 46.77% of all abnormalities, which aligns with the 44.79% reported in the current study [18]. Additionally, the study of *Ocak et al*. confirms this finding with 46% of all abnormalities [19]. Regarding structural chromosomal abnormalities, the findings of this study are also consistent with previous research. A study by *Liu et al.* reported that deletion, duplication, inversion, and translocation were the most common structural abnormalities detected prenatally [18].

The distribution of chromosomal abnormalities among the different prenatal diagnostic indications also aligns with previous studies. Advanced maternal age and abnormal serum screening have been consistently associated with higher rates of chromosomal abnormalities, particularly numerical abnormalities like trisomy 21 [20]. The higher detection rate of structural abnormalities in cases with parental chromosomal abnormalities or abnormal NIPT results has also been reported in the literature [21, 22]. The abnormal NIPT group, a new kind of prenatal screening in which fetal DNA was extracted from maternal serum for testing, had the second-highest detection rate. It is commonly used in pregnancy screening because of its excellent safety and specificity [23]. NIPT mainly targets the detection of autosomal aneuploidy, and 12 cases of autosomal aneuploidy were detected in the NIPT high-risk group, including 8 cases of trisomy 21, 3 cases of trisomy 18 and 1 case of trisomy 13. However, according to the statistical results, the high risk of NIPT also implied the possibility of mosaicism, inversion, and translocation.

Prenatal ultrasonography cannot directly detect fetal chromosomal abnormalities, but it can detect some ultrasound soft markers associated with genetic abnormalities. These soft indicators include thickened nuchal fold, echogenic focus in the heart, choroid plexus cyst and others [24]. Positive ultrasound soft indicators showed an increased risk of aneuploidy in the fetus. In our study, abnormal karyotypes were detected in 10 pregnant women with abnormal ultrasound findings, with a detection rate of 14.93% (10/67), 8 of which were aneuploid. Ultrasonography in the middle of pregnancy is essential for prenatal screening of chromosomal abnormalities, especially in fetuses with chromosomal aneuploidy.

While traditional karyotyping via amniocentesis remains the gold standard for prenatal chromosomal analysis, new technologies are emerging that offer alternatives or supplements to this invasive procedure. Non-invasive prenatal testing (NIPT) using cell-free fetal DNA from the mother's blood has become an increasingly common screening tool, with an abnormal NIPT result prompting 5.91% of the amniocenteses in this study. NIPT has high detection rates for common aneuploidies like trisomies 21, 18, and 13, though it has limitations in identifying structural chromosomal abnormalities [25]. Additionally, chromosomal microarray analysis (CMA) and next-generation sequencing (NGS) are newer technologies offering higher resolution and the capability to detect submicroscopic chromosomal alterations that karyotyping might miss [26].

In conclusion, 96 karyotype abnormalities were detected in 1285 high-risk pregnant women, with an abnormality rate of 7.47%. NIPT, ultrasound, and serological screening help detect fetal chromosomal abnormalities. Nevertheless, karyotype analysis is still irreplaceable. Karyotype analysis of amniotic fluid cells is recommended for all pregnant women with indications of prenatal diagnosis. However, this study has limitations in that only the main indications were included in the statistics for pregnant women who met several indications. The sample size was small, and more research is required in future work.

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Conflict of competence

The authors declare no conflict of interest.

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CM: Contributed to the conception of the work, data collection, conducting the study, and data analysis, and agreed to all aspects of the work. LS: Contributed to the conception of the work, conducted the study, revised the draft, approved the final version of the manuscript, and agreed to all aspects of the work. LL: Collection and entry of data, manuscript writing, translation and editing. All authors: Final approval of the manuscript.

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