Research Article

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Amniotic fluid karyotype analysis and prenatal diagnosis strategy of 3117 pregnant women with amniocentesis indication

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Aim: To examine prenatal diagnosis strategies through fetal karyotype analysis for 3117 pregnant women with genetic amniocentesis indications. **Materials & methods:** According to the different indications for amniocentesis, the study was divided into 8 groups. The number of amniocentesis specimens, the number of abnormal karyotypes and the positive rate of each group were analyzed. **Results:** Compared with prenatal serum screening, noninvasive prenatal DNA testing is more accurate and can effectively improve screening efficiency. Multiple prenatal diagnosis indicators (37.349%) were more likely to be detected than single prenatal diagnosis indicators (11.091%). **Conclusion:** None of the screening methods can completely replace amniocentesis, and for pregnant women with genetic indications for amniocentesis, amniocentesis is strongly recommended.

Plain language summary

What is this article about? This article discusses prenatal diagnosis strategies through fetal karyotype analysis for 3117 pregnant women with genetic amniocentesis indications.

What were the results? None of the screening methods can completely replace amniocentesis, and for pregnant women with genetic indications for amniocentesis, amniocentesis is strongly recommended.

What do the results of the study mean? Amniocentesis combined with karyotype analysis has long been the gold standard for the diagnosis of fetal chromosomal disorders.

Tweetable abstract: No alternative screening methods can completely replace amniocentesis, and for pregnant women with genetic indications for amniocentesis, amniocentesis is strongly recommended.

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Keywords: amniocentesis • chromosome abnormality • NIPT • non-invasive prenatal DNA testing • prenatal diagnosis

Chromosome number and morphology are not only important markers of species, but also the basis for maintaining genetic stability of species. Human chromosome abnormalities, also called chromosomal aberrations, can be divided into two categories: number abnormalities and structure abnormalities. Among them, chromosome number abnormality can be divided into euploid abnormality and aneuploid abnormality. The structural abnormalities of chromosomes mainly include deletion, duplication, inversion and translocation. Chromosomal aberrations are affected by a variety of factors such as physical, chemical, or genetic factors. Diseases caused by chromosomal abnormalities are called chromosomal disorders. Most chromosomal disorders are mental retardation, hypoplasia and multiple malformations, and there is no effective treatment at present.

Amniocentesis is the most commonly used invasive prenatal diagnostic technique. As early as the 1880s, German scientists used amniocentesis to treat the problem of excessive amniotic fluid. In the 1950s, this method has been applied in clinical fetal sex determination [1] and the diagnosis of fetal Rh hemolytic disease. Since 1966, when Steele and Breg isolated amniotic fluid cells from amniotic fluid for culture and fetal karyotype analysis [2],

amniocentesis has been widely used in prenatal diagnosis of fetal chromosomal disorders, and became the "gold standard" in invasive prenatal diagnosis in the 1980s. It is very important to diagnose chromosomal disorders before birth [3]. Karyotype analysis of amniotic fluid collected by ultrasound-guided amniocentesis is the main method for diagnosis of fetal chromosomal abnormalities [4]. Amniocentesis is an invasive prenatal diagnosis with a certain risk of abortion, so it is only applicable to pregnant women with indications for amniocentesis in clinical practice.

Due to the surgical risk of amniocentesis, it will cause certain psychological pressure on pregnant women [5]. Therefore, the search has been on for a technique that can reduce the risk. In 1997, Professor Yuming Lu of the Chinese University of Hong Kong found cell-free fetal DNA fragments (CFF-DNA) in maternal plasma, and expounded their detection significance in detail [6,7]. Researchers use next-generation high-throughput gene sequencing technology combined with modern bioinformatics analysis methods to establish noninvasive prenatal DNA testing (NIPT) for free fetal DNA fragments contained in the peripheral blood of pregnant women. The risk of fetal chromosomal aneuploidy can be determined by analyzing CFF-DNA fragments in maternal blood. Greatly improve the accuracy of prenatal screening. CCF DNA is present in the maternal blood, mainly from cells in the placenta. Non-invasive DNA detects the genetic material of the placenta, but because the placenta and the fetus come from the same fertilized egg, they usually have the same genetic material. NIPT is highly accurate, safe, simple and non-invasive, and has been widely recognized by scholars at home and abroad [8,9] and has been used in clinical practice [10,11].

Although NIPT is effective in avoiding the risks that amniocentesis can pose to the fetus and pregnant woman. However, in clinical practice, whether non-invasive prenatal DNA testing should replace invasive prenatal diagnosis is still highly controversial. In this study, the amniotic fluid karyotypes of 3117 pregnant women with indications for amniocentesis were analyzed, and the percentage and detection of abnormal karyotypes of amniocentesis with different indications were observed. This study provides a basis for genetic counseling and prenatal diagnosis strategies.

Materials & methods

Subjects

A total of 3117 pregnant women aged 19–52 years received amniocentesis at 15–33 weeks of gestation in the Prenatal Diagnosis Center of Zibo Maternal and Child Healthcare Hospital from 2017 to 2021.

Methods

Amniocentesis was performed under ultrasound guidance to collect 20 ml of amniotic fluid, which was divided into 2 tubes on average. After centrifugation at 1800 r.p.m. for 10 min, the remaining 1 ml of supernatant was removed and then inoculated in two 25 cm^2 culture bottles containing Baidi medium (Guangzhou, China). Two batches were cultured at 37°C and 5% CO_2 at the same time. Cell adherence and growth were observed on 6–7 days after inoculation, and the medium was replaced in time. After 2–3 days, the cells were harvested after 4 hours of colchicine treatment. The harvested cell suspensions were then stained with Giamsa after a drip and bake process. Zeiss automatic chromosome scanner (Imager, Z2) was used to obtain chromosome images, and chromosome karyotype analysis was performed by chromosome analysis system (MetaClient).

The amniotic fluid cell culture was successful if it could count 20 metaphase phase and analyze 5 karyotypes. Otherwise, it was failed. Karyotyping was performed according to the An International System for Human Cytogenomic Nomenclature (2020) (ISCN 2020) [12].

According to the different indications of amniocentesis, this study is divided into 8 groups: (a) Pathological ultrasound finding, PUF; (b) Advanced maternal age, AMA; (c) Prenatal serological screening tests, PSS; (d) Non-invasive prenatal DNA testing, NIPT; (e) At least one of the couple has abnormal karyotype; (f) Pregnant women with adverse pregnancy and birth history; (g) Test-tube baby; (h) Pregnant women requested an amniocentesis; the number of amniocentesis specimens, abnormal karyotypes and positive rate were observed.

Statistical Analysis

Excel was used for statistical analysis of data. Pregnant women with two or more amniocentesis indications were included in different groups for repeated statistics.

Table 1. Detection rates of abnormal karyotype in different indications of prenatal diagnosis.

Indications of prenatal diagnosis	Cases (%)	Abnormal karyotype (n)	Positive detection rate (%)
Pathological ultrasound finding	383 (12.287%)	52	13.577
Advanced maternal age	842 (27.013%)	83	9.857
Prenatal serum screening tests	1007 (32.307%)	62	6.157
Noninvasive prenatal DNA testing	760 (24.382%)	325	42.763
At least one of the couple has abnormal karyotype	115 (3.689%)	26	22.609
Pregnant women with a history of adverse pregnancy and childbirth	254 (8.149%)	4	1.575
Test-tube baby	5 (0.160%)	1	20.000
Pregnant women requested an amniocentesis	26 (0.834%)	0	0.000
Total	3117 (100%)	434	13.924

Pregnant women with two or more amniocentesis indications were included in different groups for repeated statistics.

Table 2. Abnormal karyotype in this study.

Karyotypes	Cases (n)	%
Abnormalities	**353**	**81.336**
Trisomy 21	203	46.774
Trisomy 18	38	8.756
Trisomy 13	3	0.691
Superfemale syndrome	27	6.221
Supermale syndrome	19	4.378
Klinefelter syndrome	40	9.217
Turner syndrome	19	4.378
Marker chromosome	4	0.922
Structural abnormalities	**81**	**18.664**
Translocation	23	5.300
Deletion	10	2.304
Inversion	15	3.456
Isochromosome	3	0.691
Derivative chromosome	16	3.687
Duplication	4	0.922
Other	10	2.304
Total	**434**	**100.000**

Results

In the 3117 cases, the proportion of pregnant women with PUF was 12.287% (383 cases), and the positive rate was 13.577%. The proportion of pregnant women with AMA was 27.013% (842 cases), and the positive rate was 9.857%. The proportion of pregnant women with PSS was 32.307% (1007 cases), and the positive rate was 6.157%. The proportion of pregnant women with NIPT was 24.382% (760 cases) and the positive rate was 42.763%. The proportion of pregnant women with abnormal karyotype in at least one of the couples was 3.689% (115 cases), and the positive rate was 22.609%. The proportion of pregnant women with adverse pregnancy history was 8.149% (254 cases) and the positive rate was 1.575%. The proportion of pregnant women undergoing IVF was 0.160% (5 cases), and the positive rate was 20.000%. The proportion of pregnant women requiring amniocentesis was 0.834% (26 cases), and the positive rate was 0 (Table 1).

In the 3117 cases, chromosomal abnormalities were found in 434 cases (13.924%). Among the 434 cases, 353 cases (81.336%) had abnormal chromosome number and 81 cases (18.664%) had abnormal chromosome structure. Among the abnormal chromosome number types, trisomy 21 (46.774%, 203/434) was the most significant. Chromosomal translocation (5.300%, 23/434) was the most significant among the types of chromosomal structural abnormalities (Table 2).

According to Table 3, NIPT group (335 cases) was the most positive group. AMA group (81 cases), PSS group

Table 3. Distributions of abnormal karyotype in different indications of prenatal diagnosis.

Karyotypes	Cases (n)	Indications of prenatal diagnosis							
		a	b	c	d	e	f	g	h
Trisomy 21	203	30	48	35	175	1	0	0	0
Trisomy 18	38	13	8	5	31	0	0	0	0
Trisomy 13	3	1	0	0	2	0	1	0	0
Superfemale syndrome	27	0	5	3	26	0	0	0	0
Supermale syndrome	19	1	3	2	17	0	0	0	0
Klinefelter syndrome	40	0	6	3	37	0	1	0	0
Turner syndrome	19	3	0	4	16	0	0	0	0
Marker chromosome	4	0	0	2	1	0	1	0	0
Translocation	23	1	4	4	4	13	0	0	0
Deletion	10	2	2	2	4	1	0	0	0
Inversion	15	3	2	3	2	5	0	0	0
Isochromosome	3	0	0	0	3	0	0	0	0
Derivative chromosome	16	2	1	2	7	6	0	1	0
Duplication	4	0	0	0	3	1	0	0	0
Other	10	0	2	2	7	0	0	0	0
Total	434	56	81	67	335	27	3	1	0

(a) PUF group; (b) AMA group; (c) PSS group; (d) NIPT group; (e) At least one of the couple has abnormal karyotype Pregnant women requested an amniocentesis; (f) Pregnant women with adverse pregnancy and birth history; (g) Test-tube baby group; (h) Pregnant women requested an amniocentesis.

Table 4. Distribution of abnormal karyotypes with single or multiple prenatal diagnostic indications.

	Prenatal diagnoses, n (%)	Cases (n)	%
Single factor	2795 (89.670%)	310	11.091
Multiple factors	322 (10.330%)	124	37.349

(67 cases) and PUF group (56 cases) followed. Three karyotypes, translocation (13/27), inversion (5/27) and derivative (6/27), were found in the chromosome abnormality group of at least one of the couple. Pregnant women with adverse pregnancy and birth history group and Test-tube baby group were less positive, the pregnant women requested an amniocentesis group was 0. It was more likely to be detected with multiple prenatal diagnosis indicators (37.349%) than with a single prenatal diagnosis indicator (11.091%) (Table 4).

Discussion

At present, amniocentesis combined with karyotype analysis is still the gold standard in clinical diagnosis of fetal chromosomal disorders. This method may make the fetus have a certain risk of abortion or infection, and the operation itself will also have a certain psychological pressure on pregnant women. As a result, non-invasive methods have become the trend of the industry as opposed to invasive prenatal diagnosis.

Currently, non-invasiveprenatal diagnostic technologies include prenatal serological screening, ultrasound exploration, and NIPT. Prenatal serological screening by detecting the levels of related proteins and hormones in the blood of pregnant women, using supporting analysis software combined with the history, age, weight, gestational age of pregnant women to calculate the risk of fetal chromosomal genetic diseases and other diseases. This technique screens for trisomy 21, trisomy 18 risk, and neural tube malformations. Studies have shown that the chromosomal abnormality detection rate of high-risk serological screening population is higher than that of low-risk population, but at the same time, there is a high false-positive rate. In this study, 1007 pregnant women underwent prenatal serological screening, and the positive rate was only 6.157% (62/1007). In addition to the deformity directly detected by ultrasound exploration, some studies [13] have confirmed the correlation between ultrasonic abnormality and chromosomal abnormality. First-trimester ultrasound exploration may identify most fetuses affected by structural abnormalities. Prenatal ultrasound exploration can diagnose the vast majority of fetal structural malformations, but there are some differences in the difficulty and detection time [14] of different anatomy and diseases. Some malformations are difficult to detect, and ultrasound still has technical defects. In this study, abnormal fetal karyotype was found in 52 of 383 PUF pregnant women, with a detection rate of 13.577%. Trisomy 13, 18 or 21 was

found in 44 of 52 cases. NIPT is based on the sequencing of free DNA fragments in pregnant women's peripheral blood. By comparing the reference sequence, the position information of each chromosome sequence is obtained and interpreted. At present, NIPT is widely used in clinical detection of T21, T18 and T13 with a high detection rate [15], but the detection effect of NIPT for chromosomal abnormalities other than common chromosomal aneuploidy is not good, and the positive predictive value of chromosomal microdeletion/microduplication syndrome is lower [16]. In addition, NIPT also has false negative and false positive. One study reported that NIPT failed in 2–6% of cases [17]. Another study reported a ratio of false-positive to false-negative NIPT results of 27:1 [18]. NIPT remains a pure risk assessment test designed to detect specific chromosomal abnormalities from the peripheral blood of pregnant women. It is important to emphasize that families who purchase this test and obtain normal results may have a false sense of security. NIPT is highly sensitive to CPM, which is an important cause of spurious NIPT results [19]. It should be retested by technologies such as copy number variation sequencing and single nucleotide polymorphism microarray. In this study, 325 pregnant women underwent NIPT, with a positive rate of 42.763% (325/760).

Whether non-invasive prenatal diagnostic techniques can replace traditional karyotyping remains controversial. Among the 3117 cases in this study, the detection rate of fetal chromosomal abnormalities was 13.924%, which was significantly higher than the results reported by Tseng [20] (2.9%) and Karaoguz [21] (3.0%). The most common chromosomal abnormality in newborns is trisomy 21. In this study, trisomy 21 had the highest proportion (46.77%) among the 434 fetuses with chromosomal abnormalities. Ocak *et al.* [22] also found that trisomy 21 was the most common chromosomal abnormality, accounting for 46% of 6124 amniotic fluid samples. Zhang *et al.* [23] reported that in 13,796 amniotic fluid samples, Down syndrome accounted for 35.6%. Therefore, karyotype analysis in high-risk population is of great significance. The resolution of chromosome karyotyping was only 5–10M. Abnormalities such as microdeletion/microduplication, imprinting effect, single gene or complex inheritance, and small mosaicism that cannot be detected by karyotype analysis can be further analyzed by higher resolution techniques. For example, copy number variation sequencing, single nucleotide polymorphism microarray, chromosomal microarray analysis, whole exome sequencing, whole genome sequencing and other tests are used.

Among the 3117 cases in this study, abnormal PSS accounted for 32.307% (1007 cases) and AMA accounted for 27.013% (842 cases). Pregnant women with amniocentesis indication had the most abnormal PSS, followed by the pregnant women with AMA. With the liberalization of China's three-child policy, there will be more and more elderly pregnant women. In this study, the detection rate of chromosomal abnormalities in elderly pregnant women was 9.857 (83/842), slightly higher than that in PSS pregnant women (6.157%, 62/1007). Therefore, AMA can be used as an independent indication of hereditary amniocentesis. In the process of meiosis, zygotes in elderly pregnant women are prone to chromosome non-separation, resulting in the formation of aneuploidy chromosomes. Therefore, amniocentesis is recommended for pregnant women aged 35 and over to avoid wasting medical resources. With the improvement of ultrasound resolution, more and more ultrasound soft indicators and fetal microstructural abnormalities are found before birth. The more abnormal the ultrasound fetus, the greater the likelihood of chromosomal abnormalities. Therefore, B ultrasound is an important means to prevent the birth of fetuses with chromosomal disorders. Even with the rapid development of molecular detection technology, ultrasound examination during pregnancy is indispensable, and there is still the possibility of chromosomal abnormalities and simple structural abnormalities with low risk serological and non-invasive prenatal genetic testing. NIPT is easily accepted by pregnant women because it is safe, accurate and fast. However, due to the false positives and false negatives of the NIPT technique, the test may be missed. It is well known that the cost of PSS is much lower than that of NIPT. PSS is not only of screening significance for chromosomal disorders, but also for some pregnancy complications, fetal growth restriction and adverse pregnancy outcomes, such as increased β-HCG or decreased AFP. PSS is also used as a screening test for fetal neural tube defects. Therefore, NIPT can identify trisomy 13, 18, or 21 at the molecular level and, although false-positive and false-negative rates are low, is not a substitute for PSS.

NIPT is more accurate than PSS, but neither is a complete replacement for amniocentesis. It is more likely to point to a positive result when multiple heritable amniocentesis indications are present. Neither single nor multiple indications for prenatal diagnosis can be fully diagnosed. For pregnant women with genetic indications for amniocentesis, we strongly recommend direct amniocentesis. If a pregnant woman refuses invasive prenatal diagnosis, NIPT and PSS are performed first. If NIPT and PSS results are negative, pregnant women should be followed up with PUF. However, if NIPT and PSS results are positive, invasive prenatal diagnosis should be performed.

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Conclusion

In conclusion, non-invasive and accurate diagnosis will be the development trend of prenatal diagnosis in the future. However, NIPT cannot replace PSS, B ultrasound and invasive prenatal diagnosis. Prenatal diagnosis strategies should be based on a reasonable combination of non-invasive prenatal diagnosis techniques (PSS, PUF, NIPT) and invasive prenatal diagnosis techniques.

Summary points

- Amniocentesis combined with karyotype analysis has long been the gold standard for the diagnosis of fetal chromosomal disorders.
- Compared with prenatal serum screening, noninvasive prenatal DNA testing is more accurate and can effectively improve screening efficiency.
- Multiple prenatal diagnosis indicators were more likely to be detected than single prenatal diagnosis indicators.
- None of the screening methods can completely replace amniocentesis, and for pregnant women with genetic indications for amniocentesis, amniocentesis is strongly recommended.

Author contributions

Y Liu, XC Sun and K Mu designed the study. Y Liu, XC Sun, GJ Lv, JH Liu and C Sun were responsible for cell culture and karyotype analysis. Y Liu and XC Sun prepared the manuscript. Y Liu, XC Sun and K Mu were responsible for revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

All studies were approved by the Ethics Committee of Zibo Maternal and Child Health Hospital and all patients in this study signed informed consent for amniotic fluid cell culture and chromosome karyotype analysis.

Data sharing statement

All data generated or analyzed during this study are included in this published article.

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