European Review for Medical and Pharmacological Sciences 2023; 27: 7101-7106

Analysis of the results of non-invasive prenatal testing (NIPT) in 545 pregnant women in advanced maternal age

J.-Y. SU, Y.-N. WEI, H.-F. CHEN, J.-R. TONG, Y. CHEN, L. DENG, L.-L. HUANG, L.-Y. ZHANG

Department of Obstetrics, The Second Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China

J.-Y. Su and Y.-N. Wei have contributed equally to this work and are both first authors

Abstract. – OBJECTIVE: This research aimed to explore the value of non-invasive prenatal testing (NIPT) as a prenatal screening method for common aneuploidy in pregnant women in advanced maternal age.

PATIENTS AND METHODS: A retrospective analysis was conducted on a cohort of 545 mothers with singleton pregnancy who were of advanced age and underwent NIPT testing voluntarily at the Second Affiliated Hospital of Guangxi Medical University between November 2020 and February 2023. In cases where NIPT testing suggested chromosomal abnormalities, amniocentesis was conducted, karyotype analysis or gene copy number variation (CNV) testing was performed, and the pregnancy outcome was tracked.

RESULTS: Among 545 pregnant women in advanced maternal age, 11 cases had high risk of NIPT, and the detection rate was 2.02%. Among 11 pregnant women deemed to be at high risk for NIPT, 10 cases underwent amniotic fluid puncture, and one case refused amniocentesis despite a suggestive chromosomal abnormality in NIPT. The overall rate of amniocentesis was 1.83%. Among 11 pregnant women deemed to be at high risk for NIPT, the results suggested that 5 of them had trisomy 21, 1 had trisomy 18, 2 had sex chromosome abnormalities (specifically, *47, XYY*), and 3 had other autosomal abnormalities. The positive predictive values of NIPT were 100.00% for the cases of trisomy 21 and trisomy 18, while the values were 0.00% for the cases of sex chromosome abnormalities and other autosomal abnormalities, respectively. After the follow-up, each of the 6 cases that were diagnosed with definite chromosomal abnormalities during prenatal screening opted to induce labor and terminate the pregnancy, including 5 cases that exhibited a high risk of trisomy 21 (*47, XN,+21*) and 1 case that showed a high risk of trisomy 18 (*47, XN,+18*). One instance of NIPT indicated a potential abnormality in the sex chromosomes, the individual declined to undergo amniocentesis. Another instance of NIPT suggested a sex chromosome abnormality, amniocentesis revealed a deletion of 0.72 Mb in the 4q22.1 region. They all had normal pregnancies and normal newborns. The remaining three cases had normal prenatal diagnoses (*46, XN*) and experienced normal pregnancies with healthy neonatal outcomes.

CONCLUSIONS: NIPT has demonstrated its efficacy as a screening tool in the face of increasing maternal age. As a result, it can substantially decrease the requirement for invasive prenatal diagnosis. Nonetheless, there are instances of erroneous positive outcomes in NIPT testing, and therefore, interventional prenatal diagnosis remains necessary for individuals with high-risk screening outcomes to prevent false positives or unwarranted labor induction.

Key Words:
Non-invasive prenatal testing, NIPT, Advanced maternal age, Pregnancy, Pregnancy outcomes.

Introduction

According to statistical data, birth defects affect approximately 5%-6% of newborns in China[1]. These defects cause immense suffering and financial strain on affected children and their families. Chromosomal abnormalities are a significant contributor to birth defects, and their incidence generally increases with maternal age[2]. Therefore, prenatal screening for fetal chromosomal abnormalities is crucial for pregnant women over the age of 35, as it is the most effective method

Corresponding Authors: Liying Zhang, MD; e-mail: zhangliying@gxmu.edu.cn; Lingling Huang, MD; e-mail: zigan002022@163.com

of prevention and control. Serologic marker testing and ultrasound are commonly used screening methods for birth defects. However, the detection rate for serologic markers is only between 60-85%, and there is a 5% chance of false positives[3]. Although interventional amniocentesis for fetal karyotyping and copy number variation (CNV) testing is considered the "gold standard" for confirming chromosomal abnormalities[4,5], it is an invasive procedure that carries risks such as fetal injury, teratogenicity, miscarriage, and uterine defects. Pregnant women may also experience psychological burdens and concerns regarding the invasive nature of the procedure and potential risks to their unborn child. Non-invasive prenatal testing (NIPT) is a technique that utilizes the peripheral blood of pregnant women to extract cell-free fetal DNA (cffDNA). This technique employs a new generation of high-throughput sequencing technology combined with bioinformatics analysis to calculate the relative content of cffDNA and determine the presence of fetal aneuploidy. NIPT has a higher sensitivity and specificity in detecting chromosomal abnormalities such as trisomy 21, trisomy 18, trisomy 13, and sex chromosomes compared to conventional serological tests[6,7]. Additionally, NIPT has the advantages of being non-invasive, safe, having a short testing period, and early detection, which makes it more readily accepted by pregnant women of advanced age. To evaluate the value of this screening tool in prenatal screening for common aneuploidy pregnant women in advanced maternal age, this study analyzed the results of NIPT in 545 elderly pregnant women.

Patients and Methods

Patients

The study enrolled 545 expectant women who carried singleton pregnancies and were aged 35 years or older. These women voluntarily underwent non-invasive prenatal testing (NIPT) at the Second Affiliated Hospital of Guangxi Medical University between November 2020 and February 2023. The mean age of the participants was 36.72±2.09 years, and the gestational week ranged from 35 to 49, with a mean of 17.01±2.51 weeks. The study received ethical clearance from the Medical Ethics Committee of the Second Affiliated Hospital of Guangxi Medical University, and all participants provided informed consent.

Method

NIPT Testing

10 mL of peripheral blood was extracted from expectant mothers and analyzed at the Genetic Experiment Center of the Second Affiliated Hospital of Guangxi Medical University. Fetal-free DNA was isolated from the samples and subjected to cffDNA end repair and ligation reaction to obtain double-stranded DNA molecules. Subsequently, PCR amplification was carried out to generate the DNA sequencing library. High-throughput gene sequencing technology was employed to perform sample sequencing analysis. The proportion of reads and Z-value for each chromosome were computed. A Z-value between -3 and 3 was considered low risk, a Z-value of ≥3 indicated a high risk of trisomy, and a Z-value of ≤-3 indicated a high risk of monosomy.

Chromosome karyotype and CNV testing

Pregnant women who received high-risk results in their prenatal testing underwent genetic counseling and provided informed consent for amniocentesis, which a qualified prenatal diagnosis physician performed under ultrasound guidance. Amniotic fluid was extracted for prenatal diagnostic testing to confirm the results of NIPT. The extracted cells were cultured and analyzed for karyotype using standard hypotonicity, fixation, filming, G banding, and staining treatment. The karyotype was then compared to ISCN2016 for analysis. Additionally, CNV detection was performed by isolating genomic DNA from the amniotic fluid cells and typing it into small fragments of nucleic acid. The interrupted genomic DNA was used as the starting template for library preparation, which was performed using the PCR-free library-building method. The quality-controlled DNA libraries were then sequenced on the Illumina NextseqCN500 sequencing platform to obtain the required amount of data for chromosomal abnormality analysis. Finally, the results were analyzed using the gene database.

Pregnancy Outcome Follow-up

Every pregnant woman whose NIPT test indicated a high risk was monitored. The results of the prenatal tests, the pregnancy outcomes, and the neonatal condition were documented.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 25.0 software (IBM Corp., Armonk, NY, USA) was used to analyze the data.

The proportion of chromosomal abnormalities was calculated by directly counting their frequency.

Results

NIPT Testing Results

Out of the total 545 pregnant women, 11 cases were identified as high-risk, with a detection rate of 2.02%. These included 5 cases of trisomy 21, 1 case of trisomy 18, 2 cases of sex chromosome abnormalities (*47, XYY*), and 3 cases of other autosomal abnormalities.

Comparison Between NIPT and Amniocentesis

Among 11 patients deemed high-risk for NIPT, 10 consented to undergo amniocentesis. Of these 10 patients, 6 cases of chromosomal abnormalities were detected, with 5 cases of trisomy 21 and 1 case of trisomy 18. One patient who had NIPT suggestive of sex chromosomal abnormality declined amniocentesis. And another patient with the same indication showed no karyotype abnormality but had CNV indicating 0.72 Mb deletion in the 4q22.1 region, with unknown significance. Lastly, 3 patients with NIPT suggestive of other autosomal abnormalities showed no karyotype or CNV abnormalities (Table I).

Pregnancy Outcome

Among the 11 pregnant women who underwent NIPT screening and were identified as high-risk, six proceeded with amniocentesis to confirm the results. Among these six cases, five were found to have trisomy 21, and one was diagnosed with trisomy 18, leading them to choose to terminate the pregnancy through induction of labor. One patient, who received amniocentesis results indicating a 0.72 Mb deletion in the 4q22.1 region, chose to continue the pregnancy and give birth to a healthy baby. The remaining three cases had normal amniocentesis results (*46, XN*) and delivered healthy babies (Table II).

Discussion

Chromosomal abnormalities have a significant impact on the growth, development, and cognitive abilities of newborns, placing a considerable medical and financial burden on affected children, their families, and society. As maternal age rises and ovarian function declines, the likelihood of embryonic chromosomal abnormalities increases, leading to higher rates of embryonic aneuploidy, miscarriage, and preterm delivery before 34 weeks gestation[8,9]. Therefore, conducting chromosomal screening for older pregnant women is crucial for guiding their care during pregnancy. Since most older pregnant women have valuable fetuses, they tend to reject amniocentesis due to its invasive nature and the potential risks of fetal injury, teratogenicity, miscarriage, and intrauterine infection. Instead, they seek efficient, speedy, and safe prenatal screening methods. The cffDNA can be detected in the plasma of women starting from the seventh week after conception. The concentration of cffDNA in the plasma increases over time, with higher levels observed during mid-to-late pregnancy compared to early pregnancy. Furthermore, cffDNA clears up on its own after pregnancy termination[10,11]. NIPT is a rapidly advancing technology in the field of prenatal diagnosis. It involves analyzing the ratio of cffDNA present in maternal plasma to identify fetal chromosomal abnormalities. NIPT offers several advantages over traditional invasive prenatal

Table I. Comparison between NIPT and amniocentesis.

Types of chromosomal abnormalities	NIPT High-Risk	Amniocentesis	Amniocentesis results	Positive predictive value of NIPT
Trisomy 18	5	5	All 5 cases were *47, XN,+21*	100.00%
Trisomy 18	1	1	*47, XN,+18*	100.00%
Sex chromosome abnormalities	2	1	No karyotype abnormality, CNV suggested 0.72 Mb deletion in region 4q22.1, significance unknown	0.00%
Other autosomal abnormalities	3	3	No karyotype or CNV abnormalities were found in the three cases	0.00%

NIPT: non-invasive prenatal testing; CNV: copy number variation.

Table II. Pregnancy outcomes of 11 high-risk pregnancies diagnosed through NIPT.

ID	Age	NIPT results	Amniocentesis results	Pregnancy outcome	Newborn status
1	37	Trisomy 21	*47, XN,+21*	Labor Induction	
2	38	Trisomy 21	*47, XN,+21*	Labor Induction	
3	39	Trisomy 21	*47, XN,+21*	Labor Induction	
4	40	Trisomy 21	*47, XN,+21*	Labor Induction	
5	41	Trisomy 21	*47, XN,+21*	Labor Induction	
6	38	Trisomy 18	*47, XN,+18*	Labor Induction	
7	35	Sex chromosome abnormalities	Rejection of amniocentesis	Normal labor	Normal
8	49	Sex chromosome abnormalities	4q22.1 region 0.72Mb deletion	Normal labor	Normal
9	35	Other autosomal abnormalities	*46, XN*	Normal labor	Normal
10	36	Other autosomal abnormalities	*46, XN*	Normal labor	Normal
11	37	Other autosomal abnormalities	*46, XN*	Normal labor	Normal

Body mass index (BMI).

diagnosis, including high safety, early detection, and shorter reporting timeframes. Consequently, it has gained popularity among high-risk pregnant women and their families. This study aimed to explore the value of NIPT as a prenatal screening method for common aneuploidy in pregnant women in advanced maternal age. A total of 545 cases were evaluated using NIPT, and only ten high-risk pregnant women required invasive prenatal diagnosis, resulting in an amniocentesis rate of only 1.83%. This approach effectively reduced the number of invasive prenatal diagnoses in elderly pregnant women, minimizing the risks associated with such procedures and avoiding unnecessary fetal loss. Furthermore, it helped to conserve medical and health resources.

NIPT has been shown to have high sensitivity and specificity in detecting chromosomal abnormalities such as trisomy 21, trisomy 18, trisomy 13, sex chromosome aneuploidy (SCA), and rare chromosome aneuploidy (RCA). Specifically, the overall sensitivities for these abnormalities were 99.21%, 100.00%, 100.00%, 98.55%, and 100.00%, respectively, while the specificities were 99.95%, 99.94%, 99.98%, 99.69%, and 99.92%, respectively[12]. A study by Suzumori et al[13] reported a detection rate of 90.10% and a false positive rate of 0.21% for trisomy 21 abnormalities using NIPT. In this study, the positive predictive value of NIPT for trisomy 21 and trisomy 18 was 100.00%. After confirmation by interventional prenatal diagnosis, five pregnant women with trisomy 21 and one with trisomy 18 chose to terminate their pregnancies. This suggests that NIPT has high positive predictive value and accuracy in screening for trisomy 18 and trisomy 21 syndrome and is a valuable screening tool. However, in one case where NIPT suggested sex chromosome abnormalities, amniocentesis results showed a deletion of 0.72 Mb in region 4q22.1. In the other three cases where NIPT presented other autosomal abnormalities, amniocentesis results showed no abnormalities in karyotype and CNV. The positive predictive value for SCA and RCA was 0.00%, possibly due to the small sample size and the fact that this study targeted older pregnant women. This also indicates the limitations of NIPT in detecting chromosomal inversion, translocation, microdeletion, microduplication and other abnormalities.While NIPT is a non-invasive prenatal diagnostic tool superior to conventional serological testing, it cannot completely replace interventional karyotyping, gene microarray, and other diagnostic methods. However, it can effectively reduce the risk of invasive prenatal diagnosis in pregnant women of advanced age. In addition, NIPT showed a high degree of agreement with amniocentesis fetal karyotyping when detecting trisomy 21 and trisomy 18. NIPT in older pregnant women can effectively detect trisomy 21 and 18-trisomy syndrome, greatly reduce invasive procedures, relieve the anxiety of most pregnant women, and reduce the risk of intrauter-

ine infection and abortion. NIPT has the advantages of non-invasive, safety and short detection cycle, which is the development trend of prenatal screening and detection in the future.

Conclusions

In conclusion, NIPT has demonstrated a high accuracy and detection rate in identifying fetal trisomy 21 and 18. This technology significantly reduces the need for invasive prenatal diagnosis, making it a more acceptable option for older pregnant women. Furthermore, NIPT proves to be a superior screening tool for older pregnant women. However, it is essential to note that the study's sample size is limited, and the detection indexes require further exploration and analysis. It is also crucial to acknowledge that false-positive results may still occur with NIPT, and individuals with high-risk screening should undergo interventional prenatal diagnosis to avoid unnecessary induction of labor or false-positive results.

Acknowledgments
We would like to thank all patients who have made genuine contributions to the article and who endorse the data and conclusions, as well as the three fundings which gave us financial support: Youth Science Foundation of Guangxi Medical University, the Research Project of Health Commission of Guangxi Zhuang Autonomous Region and Guangxi Medical and health key cultivation discipline construction project.

Funding
This research was supported by Youth Science Foundation of Guangxi Medical University [grant number GXMUYSF202222] and The Research Project of Health Commission of Guangxi Zhuang Autonomous Region [grant number Z-A20220571].

Ethics Approval
This study was approved by the Second Affiliated Hospital of Guangxi Medical University Ethics Committee on 24/09/2021 [2020-KY (0147)].

Informed Consent
The patients signed informed consent.

Authors' Contributions
Junyou Su and Yanni Wei contributed to the conception and design of the study; Hongfei Chen and Junru Tong contributed to the acquisition of data; Chen Yan contributed to the analysis of data; Li Deng and Lingling Huang drafted the article; Liying Zhang made critical revisions related to the relevant intellectual content of the manuscript.

Conflict of Interest
The authors have no conflict of interest to declare.

ORCID ID
Junyou Su: 0000-0002-1820-2298
Yanni Wei: 0000-0001-7892-4682
Hongfei Chen: 0000-0002-7606-6431
Junru Tong: 0000-0003-4257-2245
Yan Chen: 0000-0003-1967-629X
Li Deng: 0000-0002-0148-6861
Lingling Huang: 0000-0002-0454-2742
Liying Zhang: 0000-0003-1314-2895

Data Availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1) Fu J, Guo JC, Xiao JP, Zhang J, Zhou L, Zhang H, Guo YX, Hou HL, Li X. The clinical application of noninvasive prenatal DNA screening in fetal chromosome aneuploidy genes detection. Chinese Journal of Family Planning 2018; 26: 1064-1067+1071.
2) Cuckle H, Morris J. Maternal age in the epidemiology of common autosomal trisomies. Prenat Diagn 2021; 41: 573-583.
3) Yamada T, Sekizawa A, Fujii Y, Hirose T, Samura O, Suzumori N, Miura K, Sawai H, Hirahara F, Murotsuki J, Kamei Y, Sago H; Japan NIPT consortium. Maternal age-specific risk for trisomy 21 based on the clinical performance of NIPT and empirically derived NIPT age-specific positive and negative predictive values in Japan. J Hum Genet 2018; 63: 1035-1040.
4) Huang LL, Chen HF, Huang Y, Wei YN, Tong JR, Chen Y, Luo J, Liao S, Wei LL, Deng L, Su JY. Analysis results of 579 cases of genomic copy number variation sequencing of pregnant women in prenatal diagnosis. Eur Rev Med Pharmacol Sci 2022; 26: 7572-7579.
5) Wang J, Chen L, Zhou C, Wang L, Xie H, Xiao Y, Zhu H, Hu T, Zhang Z, Zhu Q, Liu Z, Liu S, Wang H, Xu M, Ren Z, Yu F, Cram DS, Liu H. Prospective chromosome analysis of 3429 amniocentesis samples in China using copy number variation sequencing. Am J Obstet Gynecol 2018; 219: 287.e1-287.e18.
6) Chen YP, He ZQ, Shi Y, Zhou Q, Cai ZM, Yu B, Wang T. Not all chromosome aberrations can be detected by NIPT in women at advanced maternal age: A multicenter retrospective study. Clin Chim Acta 2018; 486: 232-236.
7) Liang D, Cram DS, Tan H, Linpeng S, Liu Y, Sun H, Zhang Y, Tian F, Zhu H, Xu M, Wang H, Yu

F, Wu L. Clinical utility of noninvasive prenatal screening for expanded chromosome disease syndromes. Genet Med 2019; 21: 1998-2006.

8) Yang L, Tao T, Zhao X, Tao H, Su J, Shen Y, Tang Y, Qian F, Xiao J. Association between fetal chromosomal abnormalities and the frequency of spontaneous abortions. Exp Ther Med 2020; 19: 2505-2510.

9) Frederiksen LE, Ernst A, Brix N, Braskhøj Lauridsen LL, Roos L, Ramlau-Hansen CH, Ekelund CK. Risk of Adverse Pregnancy Outcomes at Advanced Maternal Age. Obstet Gynecol 2018; 131: 457-463.

10) Sylvester-Armstrong KR, Rasmussen SA, Shoraka M, Hendrickson JE, Abu-Rustum RS. Fetal sex discordance between noninvasive prenatal screening results and sonography: A single institution's experience and review of the literature. Birth Defects Res 2020; 112: 339-349.

11) Soltani M, Nemati M, Maralani M, Estiar MA, Andalib S, Fardiazar Z, Sakhinia E. Cell-free fetal DNA in amniotic fluid supernatant for prenatal diagnosis. Cell Mol Biol (Noisy-le-grand) 2016; 62: 14-17.

12) Liu S, Chang Q, Yang F, Xu Y, Jia B, Wu R, Li L, Yin A, Chen W, Huang F, Yang X, Li F. Non-invasive prenatal test findings in 41,819 pregnant women: results from a clinical laboratory in southern China. Arch Gynecol Obstet 2023; Epub ahead of print. DOI: 10.1007/s00404-022-06908-3.

13) Suzumori N, Sekizawa A, Takeda E, Samura O, Sasaki A, Akaishi R, Wada S, Hamanoue H, Hirahara F, Kuriki H, Sawai H, Nakamura H, Yamada T, Miura K, Masuzaki H, Yamashita T, Kamei Y, Namba A, Murotsuki J, Tanemoto T, Fukushima A, Haino K, Tairaku S, Matsubara K, Maeda K, Kaji T, Ogawa M, Osada H, Nishizawa H, Okamoto Y, Kanagawa T, Kakigano A, Endo M, Kitagawa M, Ogawa M, Izumi S, Katagiri Y, Takeshita N, Kasai Y, Naruse K, Neki R, Masuyama H, Hyodo M, Kawano Y, Ohba T, Ichizuka K, Nagamatsu T, Watanabe A, Nishikawa N, Hamajima N, Shirato N, Yotsumoto J, Nishiyama M, Koide K, Hirose T, Sago H. Classification of factors involved in non-reportable results of noninvasive prenatal testing (NIPT) and prediction of success rate of second NIPT. Prenat Diagn 2019; 39: 100-106.