Efficiency of Non-invasive Prenatal Screening in Pregnant Women at Advanced Maternal Age

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Research article

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Abstract

Background

To evaluate the efficiency of noninvasive prenatal screening (NIPS) in the prenatal screening for fetal aneuploidies in pregnant women at advanced maternal age (AMA).

Methods

From February 1, 2015, to December 31, 2018, 29,343 pregnant women at AMA underwent NIPS and followed-up were recruited. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for detecting fetal chromosomal aneuploidies were analyzed. The relationship between maternal age and common fetal chromosomal aneuploidy was observed.

Results

The sensitivity, specificity, PPV, NPV of NIPS for detecting fetal trisomy 21 were 99.11%, 99.96%, 90.98%, and 100%, respectively. These same parameters for detecting fetal trisomy 18 were 100%, 99.94%, 67.92%, and 100%, respectively. Finally, these parameters for detecting trisomy 13 were 100%, 99.96%, 27.78%, and 100%, respectively. The prevalence of fetal trisomy 21 increased with maternal age. There were significant differences in the prevalence of fetal trisomy 21 among the groups I (35-37-year-old), II (38-40-year-old) and III (41-year-old and older) ($P < 0.05$).

Conclusion

It is indicated that NIPS is an effective prenatal screening method in pregnant women at AMA.

1. Background

With the implementation of the two-child policy in China, the number of pregnant women at advanced maternal age (AMA) has increased dramatically[1]. It has been shown that the pregnant women at AMA account for 33.4%-46% in prenatal diagnosis centers[2–5]. The risk for fetal aneuploidies increases with maternal age. Invasive prenatal diagnosis should be recommended for women at AMA in some countries including China. However, the invasive prenatal diagnosis is not accepted by some pregnant women. Non-invasive prenatal screening (NIPS) is the alternative for these women.

Cell-free fetal DNA-based NIPS has been proven to be of high sensitivity and specificity for detecting common chromosomal aneuploidies (trisomy 21, trisomy 18 and trisomy 13), with low false positive and false negative rates. Moreover, clinical observations have indicated that NIPS has excellent performance in either high-risk or low-risk population of serological screening, and the detection efficiency is much

higher than that of serological screening[6–9]. However, the application of NIPS in the prenatal screening of women at AMA is still rare, especially in Chinese population.

In this study, 29,343 AMA pregnant women (35 years of age or over) were recruited. All of them underwent NIPS and were followed-up. We intended to explore the clinical significance of NIPS to detect trisomies 21, 18 and 13 in AMA pregnant women and to provide an appropriate prenatal screening program for AMA pregnant women.

2. Subjects And Methods

2.1 Subjects

From February 1, 2015, to December 31, 2018, 29,343 AMA pregnant women (35 years old or older) who underwent NIPS and completed their pregnancy outcome follow-up at Jiaxing Maternal and Child Health Hospital and Women's Hospital, Zhejiang University School of Medicine were recruited. The maternal age ranged from 35 to 55 years of age with a median of 37, and 151 cases were 45 years of age and over. The gestational age ranged from 12 to 30 weeks, and the median was 16 weeks. The gestational age was calculated according to their last menstruation period and verified by ultrasound in early pregnancy. The pregnancy outcomes were followed-up by checking the prenatal diagnosis database, delivery and infant record of hospital or registration system, or followed-up approximately three months after deliveries. The use of the data was approved with the Institutional Review Board and all participants gave their informed consent.

2.2 NIPS

The venous blood of pregnant women was collected and stored in blood collection tubes supplemented with EDTA at 4°C. The plasma DNA was extracted and used for library construction. The fetal cell-free DNA fragments in the plasma were analyzed using the BGISEQ-100 sequencing platform. The rates of risk for fetal trisomy 21, trisomy18, and trisomy13 were obtained through bioinformatics analysis[10, 11]. The normal range of the Z score was between − 3.0 and 3.0.

2.3 Fetal karyotyping

Fetal karyotyping was recommended for women who were identified to be at high risk using NIPS. The exact process of fetal karyotyping was described previously[12].

2.4 Statistics

SPSS (SPSS Company, Chicago, IL, USA) version 19.0 software was used. Data were compared using the chi-square test, and $P < 0.05$ was used to indicate significant differences. The sensitivity, specificity, PPV, and NPV of NIPS were also calculated.

3. Results

3.1 NIPS results

29,343 women at AMA who underwent NIPS and completed their pregnancy outcome follow-up were enrolled (Table 1). A total of 145 pregnant women were identified as being high-risk for fetal trisomy 21. Among them, 111 cases were confirmed to carry fetuses of trisomy 21. Eleven were false positive. Twenty-three cases did not receive diagnosis. Among them, included was one case of spontaneous abortion due to premature rupture of membranes, three of stillbirth, five termination of pregnancies (TOP) due to fetal abnormalities (two cases of cardiac abnormalities, three cases of multiple malformations indicated), 13 of TOP without diagnosis, and one case who refused prenatal diagnosis but delivered a baby of leukemia without karyotyping. Moreover, one case was determined to be a false negative. In this 39-year-old pregnant woman, NIPS was conducted at gestational age of 13 weeks and a negative result was obtained. Ultrasound revealed fetal edema and complete endocardial cushion defect. The pregnancy was terminated after fetal trisomy 21 was confirmed.

A total of 70 pregnant women were identified to be at high risk for trisomy 18 (Table 1). Among them, 53 cases received diagnosis and 36 cases were confirmed to carry fetuses of trisomy 18. In addition, 17 cases did not receive the prenatal diagnosis, including three cases of stillbirth, 11 TOP due to fetal malformations (eight of multiple malformations, three of cardiac abnormalities), and three TOP without diagnosis.

Moreover, a total of 22 pregnant women were identified to be at high risk for trisomy 13 (Table 1). Among them, 18 cases received diagnosis and five were determined to carry fetuses of trisomy 13. Thirteen were false positive. Four cases did not receive the prenatal diagnosis and terminated their pregnancies, including three cases of multiple malformations, and one who refused diagnosis.

Table 1: Screening results of common fetal chromosomal aneuploidies

Trisomy	No. of cases with high-risk results	TP	FP	No. of cases without prenatal diagnosis	FN
T21	145	111	11	23	1
T18	70	36	17	17	0
T13	22	5	13	4	0

Data are presented as n (%), unless otherwise indicated. TP (true positive), trisomy is verified; FP (false positive), trisomy is incorrectly classified as trisomy; FN (false negative), trisomy is incorrectly classified as normal.

3.2 Detection efficiency in AMA pregnant women

In order to estimate the detection efficiency of NIPS, we focused on the sensitivity, specificity, PPV, and NPV of trisomies 21, 18 and 13. As shown in Table 2, the sensitivity, specificity and NPV for NIPS for fetal trisomy 21, trisomy 18 and trisomy 13 were all over 99%. And, the PPV for fetal trisomy 21, trisomy 18 and trisomy 13 were 90.98%, 67.92%, and 27.78%, respectively.

Table 2: Detection efficiency of common chromosomal aneuploidies by NIPS

Trisomy	Sensitivity	Specificity	PPV(95% CI)	NPV(95% CI)	Rate of TP
T21	99.11 (94.62-99.99)	99.96 (99.93-99.98)	90.98 (84.08-95.19)	100 (99.98-100)	0.382
T18	100 (88.53-100)	99.94 (99.91-99.97)	67.92 (53.55-79.70)	100 (99.98-100)	0.123
T13	100 (51.09-100)	99.96 (99.92-99.98)	27.78 (12.17-51.20)	100 (99.98-100)	0.017

Data are presented as n (%), unless otherwise indicated. PPV, positive perspective value; NPV, negative perspective value; CI, confidence intervals.

3.3 The correlation between the incidence of fetal trisomy 21 and maternal age

As shown in Table 3, the rates of high-risk and true positive for fetal trisomy 21 were positively correlated with maternal age ($P < 0.01$). Then, we classified the subjects into three groups (I: 35–37 years old; II: 38–40 years old; III: 40 years old and over). Both the high-risk rate and true positive rate for trisomy 21 were significantly different ($P < 0.05$) between groups I and II. The high-risk rate differed significantly ($P < 0.05$) between groups II and III.

Table 3: High-risk rate and true positive percentage of fetal trisomy 21 in pregnant women in different age groups

Age(y)	No. of cases	No. of cases with high-risk	Rate of cases with high-risk	TP	Rate of TP
35	7044	19	0.27	16	0.23
36	6557	26	0.40	16	0.24
37	4921	16	0.33	13	0.26
38	3824	28	0.73	21	0.55
39	2669	8	0.30	6	0.22
40	1863	12	0.64	10	0.54
41	1044	17	1.63	14	1.34
42	672	6	0.89	6	0.89
43	389	6	1.54	4	1.03
≥44	330	7	2.12	5	1.52

Data are presented as n (%), unless otherwise indicated. TP (true positive), trisomy is verified.

4. Discussion

In this study, we confirmed that NIPS had a high sensitivity, specificity and NPV for detecting trisomies 21, 18 and 13 in AMA pregnant women. In addition, the high-risk rates and incidence of trisomy 21 increased with maternal age. These findings point to the clinical significance of NIPS to detect trisomies 21, 18 and 13 in AMA pregnant women and may help doctors and pregnant women to choose a suitable prenatal screening and diagnosis way.

Serological screening is widely used for fetal aneuploidies. At present, as for the high-risk pregnant women, fetal karyotyping with amniotic fluid cells or cord blood cells is used as the diagnosis for fetal chromosomal abnormalities. In China, all AMA pregnant women are advised to undergo prenatal diagnosis. However, with the implementation of the two-child policy, the number of AMA pregnant women has increased significantly[1], which has greatly increased the demand for prenatal diagnosis[13]. However, amniotic fluid sampling or umbilical cord blood collection are invasive procedures, with the risk of miscarriage which was estimated at 0.5 to 1.0%[14, 15]. There is also a risk of infection in such procedures[16]. As a result, the overall utilization rate of both methods is low. Moreover, some pregnant women may have contraindications for invasive prenatal diagnosis, such as the high risk of inducing abortion, fever, increased tendency for bleeding, and infection[17]. Therefore, it is needed to find prenatal screening methods that better meet the clinical needs.

NIPS is a noninvasive prenatal screening technique for fetal aneuploidies. NIPS is based on high-throughput sequencing to detect cell-free fetal DNA (cffDNA) in maternal peripheral blood. In 1997, Lo et al.[18] found cffDNA in maternal blood and revealed that cffDNA was suitable for prenatal examination. However, it was not widely applied in clinic until the emergence of high-throughput sequencing [19]. Bianchi et al [20] compared NIPS and serological screening in general population, which recruited 1,914 women with singleton pregnancies from 21 centers in USA. Each sample was tested by both methods.

The positive predictive values for NIPS and standard screening were 45.5% and 4.2% for trisomy 21, and 40.0% and 8.3% for trisomy 18, respectively. NIPS showed significantly better performances than serological screening. Meanwhile, Bianchi et al [20] also found that the false negative rates were 0.3% and 0.2% for trisomies 21 and18 as detected by NIPS, respectively, which were much lower than those of serological screening (3.6% and 0.6%, respectively). Similarly, in a study of 146,958 women [21], it revealed that the sensitivity was 99.17%, 98.24% and 100%, that the specificity was 99.95% ,99.95% and 99.96%, that the PPV was 92.19%, 76.61% and 32.84%, and that the NPV was 99.99%, 100% and 100%, for trisomies 21,18 and 13, respectively. Using expanded noninvasive prenatal screening ("NIPS-Plus"), which is considered a better screening method than usual NIPS, Liang [22] demonstrated that the PPVs were 95%, 82% and 46%, for trisomies 21, 18 and 13, respectively. Those findings obtained from large size of general populations were consistent with the results of ours, indicating NIPS is suitable for pregnant women at AMA.

Lots of investigations demonstrated that NIPS is superior to serological screening and suitable for the detection of trisomy 21, trisomy 18 and trisomy 13 in all high risk or low risk populations, AMA or not[20–22]. Thus, the International Society for Prenatal Diagnosis (ISPD), the American College of Obstetricians and Gynecologists (ACOG), the Royal College of Obstetricians and Gynecologists (RCOG), and the American College of Medical Genetics and Genomics (ACMG), have recommended NIPS as the preferred screening method for all pregnant women. Additionally, NIPS has been included in a national policy or national program in 14 European countries [23]. Considering the excellent efficiency of NIPS, NIPS could be promoted as the preferred screening method for AMA pregnant women. However, invasive screening methods such as amniotic fluid analysis and cord blood collection are still needed to carry out karyotype analysis for high-risk women identified by NIPS.

Since maternal age is closely associated with the incidence of fetal chromosomal abnormalities [24], we also studied the correlation between maternal age and the incidence of trisomy 21. Generally, the incidence increased with maternal age. This is consistent with previous report[25].

We noted some shortcomings of this study. On the one hand, there were some cases without diagnosis in the high-risk population detected by NIPS. Many of cases might have had fetal aneuploidies, especially those with imaging abnormalities or fetal death. Therefore, the positive predictive values of fetal trisomy 21, trisomy 18, and trisomy 13 detected by NIPS were likely to be higher than what were described here. On the other hand, the low incidence of trisomy 18 and trisomy 13 made it impossible to carry out an age stratification study as was done for the trisomy 21. Multicenter studies with larger sample sizes are expected in the future and that should provide additional data in support of optimizing prenatal screening and diagnosis strategies for AMA pregnant women.

5. Conclusions

In summary, by analyzing the data from 29,343 AMA pregnant women, we have found that NIPS has good detection efficiency and it is suitable for screening for fetal aneuploidy in AMA pregnant women.

Abbreviations

NIPS: Noninvasive prenatal screening; AMA:Advanced maternal age; PPV:Positive predictive value; NPV:Negative predictive value; TOP:Termination of pregnancies; TP:True positive; FP:False positive; FN:False negative; cffDNA:Cell-free fetal DNA; CI:Confidence intervals; ISPD:International Society for Prenatal Diagnosis; ACOG:American College of Obstetricians and Gynecologists; RCOG:Royal College of Obstetricians and Gynecologists, ACMG:American College of Medical Genetics and Genomics

Declarations

Availability of data and materials

The data used or analyzed during the current study are included within the article.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Jiaxing Maternal and Child Health Hospital. All participants gave their informed consent in this study.

Consent for publication

Not applicable.

Competing interests

The authors have no conflicts of interest to declare.

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Authors' contributions

ZH and JXX collected clinical data and analyzed the data, ZWH and LXD performed the NIPS and karyotype analysis, XYQ was a major contributor in drafting and revising the manuscript, JJL followed up the patients, QYQ took a plan for the research, DMY designed the work and revised the manuscript. All authors read and approved the final manuscript.

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