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Authors: Yao Chen, Fang Yang, Xuan Shang, Siping Liu, Meng Li, Mei Zhong

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A study on non-invasive prenatal screening for the detection of aneuploidy

Yao Chen[1], Fang Yang[1], Xuan Shang[2], Siping Liu[1], Meng Li[3], Mei Zhong[1]

[1]*Department of Obstetrics and Gynecology, The First School of Clinical Medicine, Southern Medical University, China*

[2]*Department of Medical Genetics, School of Basic Medical Sciences, Southern Medical University, China*

[3]*Department of Obstetrics and Gynecology, The Seventh Affiliated Hospital of Southern Medical University, China*

Corresponding author:

Mei Zhong

Department of Obstetrics and Gynecology, The first school of clinical medicine, Southern Medical University, NO. 1023, Shatai South Road, Baiyun District,Guangzhou, China

phone: +8613760453426

e-mail: zhongmeidr@protonmail.com

ABSTRACT

Objectives: To explore the feasibility of clinical application of non-invasive prenatal screening to detect aneuploidy diseases.

Material and methods: A total of 14,574 singleton pregnant women who underwent Non-invasive prenatal testing (NIPT) in the Southern Hospital from 2015 to June 2017 were selected, and 6471 pregnant women with twin pregnancy who underwent NIPT in the laboratory of Bei Rui He Kang Southern Hospital from June 2016 to October 2017 were included in this study. We analyzed NIPT screening efficiency (sensitivity, specificity) in twin pregnancies and singleton pregnancies, compared the positive detection rate of NIPT in patients with or without clinical

symptoms. All NIPT high-risk results were validated by karyotyping, which were further verified by the follow-up physical examination of the neonatal.

Results: A total of 68 cases of twin pregnancy abnormalities were detected by NIPT, including 18 cases of trisomy 21, 6 cases of trisomy 18, 1 case of trisomy 13, 39 cases of Spinocerebellar ataxias (SCAs), and 4 cases of other chromosomal abnormalities. The sensitivity for trisomy 21, 18, and 13 and sex chromosome abnormality was 100%; the specificity for trisomy 21, 18, and 13 and sex chromosome abnormality was 99.97%, 99.95%, 99.97%, and 99.91% respectively. The screening efficiency was similar to that of singleton pregnancy, indicating that the NIPT technology in our laboratory for screening for aneuploidy diseases in twin pregnancy has reached the accuracy level of singleton pregnancy screening. There was a statistical difference between the risk group and the non-risk group in pregnant women with singleton pregnancy. The screening efficiency of NIPT was higher in pregnant women in the risk group, which implies that the clinical application of NIPT is inclined to detect high-risk group.

Conclusions: Non-invasive prenatal testing (NIPT) is a rapid and safe screening method with high efficiency. Non-invasive prenatal testing (NIPT) is used for the screening of aneuploidy in twin pregnancy. The efficiency is similar to that of singleton pregnancy, indicating the feasibility of clinical application. However, the efficiency of NIPT screening tends to favor the detection in high-risk groups.

Key words: twin pregnancy; non-invasive prenatal test (NIPT); chromosomal aneuploidy

INTRODUCTION

With the implementation of the second-child policy and the application of assisted reproductive technology, 20–30% of live births related to assisted reproductive technology are twins, and the number of twin pregnancies is increasing rapidly [1]. Traditional serological screening for singleton pregnancy has a better performance than twin pregnancy, so there is an urgent need for a reliable twin pregnancy screening method [2]. Non-invasive prenatal test (NIPT) is a method using massively parallel sequencing technology to sequence free fetal DNA fragments in maternal plasma. Previous studies have shown that NIPT has high sensitivity and specificity for trisomy 21, 18, 13 and sex chromosome abnormalities in singleton pregnancies

[3]. However, there is a very limited studies applying NIPT to twin pregnancy screening, especially the ones with large sample size [4]. Multiple health organizations are calling for the need of NIPT in twin pregnancy screening for aneuploidy research [5–7]. This study aimed to describe the effectiveness of NIPT in detecting aneuploidy in 6471 cases of twin pregnancy, and to provide statistical basis for the feasibility of NIPT in clinical detection of aneuploidy in win pregnancy.

MATERIAL AND METHODS

Research subjects

From 2015 to 2016, a total number of 6471 pregnant women who carried twin pregnancies in Bei Rui He Kang Southern Hospital database. The inclusion criteria: gestational week of blood collection 12–24 weeks (median gestational week 16.83 weeks); early pregnancy ultrasound determined to be double Fetal pregnancy; pregnant woman weight ≤ 100 kg; no history of chromosomal abnormalities in both spouses; no allogeneic blood transfusion, transplantation, cell therapy or immunotherapy within one year. This study has been approved by the Ethics Committee of Southern Medical University, and all pregnant women who participated in the study signed an informed consent form.

Methods

After genetic counseling, pregnant women with twins voluntarily chose NIPT. When the result was high-risk, invasive prenatal diagnosis was further performed for verification. If the result was low-risk, follow-up examination was performed regularly until the newborn was born. If the ultrasound results were abnormal, an invasive prenatal diagnosis was performed to confirm the chromosome karyotype, and the newborn was subject to routine physical examination.

Procedures

NIPT high-risk results were subject to cell chromosome karyotype analysis and verification, or first interrogating the newborn's physical examination record and then completed the chromosome karyotype test to clarify chromosomal abnormalities with the consent of the parents; all non-invasive prenatal screenings were independently completed on the experimental

platform of the Bei Rui He Kang Southern Hospital, and the karyotype results were independently examined in the cytogenetic laboratories; the low-risk results were followed up by telephone interrogation.

Non-invasive prenatal screening and data analysis

1) Sample extraction: extract 10 mL of peripheral blood from pregnant women, centrifuge at 1600 rpm for 10 min at 4℃, and distribute the supernatant to 2.0 mL centrifuge tubes; 2) Automated library construction: Extract DNA with magnetic beads, fill in the ends, connect the adapters, 3) Library purification: magnetic bead purification; 4) Library quality inspection: automated fluorescence quantitative PCR for library quality inspection, quality control qualified libraries were automatically pooled to obtain the mixed library required for sequencing, and then proceed to the next step of on-machine sequencing using sequencing platform: illumina NextSeq CN 500; 5) Data analysis: transfer the computer data to the Bebian data analysis system, perform sequence comparison and statistical analysis of the data, and obtain the Z value. The cutoff for the positive was $Z = 3.0$, when the absolute value of Z less than 3, the risk of chromosome aneuploidy was considered low; and the absolute value of Z was greater than 3, and the risk of chromosome aneuploidy was considered high.

Verification of non-invasive prenatal screening results

All high-risk results were verified by chromosomal karyotyping. If the patient had not undergone prenatal diagnosis, the conditions of the newborn was followed up by telephone until delivery. The electronic record of the medical conditions was also checked, and the type of aneuploidy was determined according to the physical examination of the neonatologist. In other cases, the karyotype results of the newborn were re-collected.

The follow-up

The follow-up of the newborns was jointly completed by Southern Hospital and Bei Rui He Kang Southern Hospital. The content of the follow-up included the content of color Doppler ultrasound structure screening during pregnancy, and abnormal appearance, development, and intellectual development of the newborn after birth. Because the aneuploidy disease also displayed typical characteristics in the appearance, it can be judged whether it is a true positive case through the physical examination of the newborn.

Statistical analysis

SPSS22.0 statistical software was used for statistical analysis. Quantitative data were expressed as mean ± standard deviation, and count data were expressed as rate. The sensitivity, specificity, false positive rate and other indicators of non-invasive prenatal screening were calculated.

RESULTS

Results of NIPT

Demographic information is as follow: in the total 6471 cases of twin pregnancy pregnant women, the average age of pregnant women was 30.91 years, of which the percentage of samples ($\geq$ 35 years old) is 24.32%, and the percentage of samples (< 35 years old) was 75.68%. The average gestational week of blood sampling for pregnant women was 16.83 weeks. A total of 68 abnormalities were detected, including 18 cases of trisomy 21, 6 cases of trisomy 18, 1 case of trisomy 13, 39 cases of Spinocerebellar ataxias (SCAs), and 4 cases of other chromosomal abnormalities (Tab. 1).

The sensitivity of NIPT for the detection of the three major chromosomal aneuploidies of twin pregnancy is 100%, the specificity is above 99%, and the highest false positive rate is 0.05%. Compared with the NIPT used in the screening of the three major chromosomal aneuploidies in singleton pregnancies, screening results in twins were more sensitive (100%/96.6%) and showed the same specificity (99.9%/99.9%) as the results of singleton pregnancy. Positive predictive value (PPV) for twins was much lower (78.1% / 90.6%).

The positive rate (0.86%) of the three major chromosomes (13, 18, 21) of pregnant women in the risk group was higher than that in the non-risk group (0.47%), and the difference was statistically significant. Binomial test: The total positive rate of the test was 0.76%, which was not significantly different from the national positive rate (0.75%) (p = 0.448).

DISCUSSION

Non-invasive prenatal testing (NIPT) has a good performance in single fetal aneuploidy [8]. A current meta-analysis of NIPT for twin pregnancy showed that: T21 detection has a sensitivity 99% and a specificity 100%, T18 has a sensitivity 85% and a specificity 100%, the screening performance of T13 cannot be judged since there was only three cases being detected

[9]. The sensitivity of traditional early pregnancy serological screening + Nuchal translucency (NT) combined screening for T21 in twin pregnancy is 89.3% and specificity is 94.6%. Although the screening results are acceptable [10], However, there are also reports that under the same false positive rate, combined screening in the first trimester does not have a higher detection rate than NT screening alone, and it also increases the economic burden of the parents [11]; The second trimester serological screening is used for the detection rate of twin pregnancy aneuploidy diseases and has a high false positive rate. It cannot provide screening for trisomy 13. Therefore, it is not recommended that the second trimester serological screening be used solely for the detection of aneuploidy in twin pregnancies [12]. Serological screening in the first trimester combined with NT and serological screening in the second trimester is an optional program, but more prospective studies are needed for validation [13]. Therefore, the screening of aneuploidy in twin pregnancy, especially for high-risk pregnant women with difficulty in pregnancy, assisted reproductive pregnancy, advanced age, and with high risk of miscarriage, demands for a non-invasive, accurate and simple method.

This study provides a clinical basis for the feasibility of non-invasive prenatal screening for twin pregnancy. The sensitivity of NIPT for the detection of the three major chromosomal aneuploidies of twin pregnancy is 100%, the specificity is above 99%, and the highest false positive rate is 0.05%, which is consistent with the results published in previous studies [14, 15]. Compared with the NIPT used in the screening of the three major chromosomal aneuploidies in singleton pregnancies, screening results in twins were more sensitive (100%/96.6%) and showed the same specificity (99.9%/99.9%) as the results of singleton pregnancy. Positive predictive value (PPV) for twins was much lower (78.1%/90.6%). The screening result of twin pregnancy in this study showed consistent performance with that in singleton pregnancy, which achieved high sensitivity and specificity that are superior to traditional serological screening. However, the positive predictive value of the test for aneuploidy in twins is lower, which may be due to the variable factors such as fetal DNA ratio of twins, chronicity and laboratory technical operations. In fact, the DNA concentration of normal fetuses in twins is higher, which can easily mask abnormal fetal DNA and lead to false results. In 2013, Professor Liang Deyang applied Single Nucleotide Polymorphism (SNP) technology to determine the genomic regions of fraternal twins and derived the proportion of each fetus's DNA to estimate the aneuploidy risk of each fetus.

However, this study has a small sample size and future work involving a larger sample size is required to validate the findings [14–16].

In addition to T21, T18, and T13, we also evaluated the effectiveness of NIPT in detecting other chromosomal aneuploidies. In the detection of twins' sex chromosomes, NIPT has achieved high detection efficiency like that of T21, T18, and T13, even higher than that of singletons. It may be because the number of sex chromosomal aneuploidy cases in this study is too low to accurately assess the efficiency of NIPT screening. Zhang, et al. [17], reported that the overall positive predictive value of NIPT for SCA was 54.54%, and Turner syndrome (45, X) was 29.41%. Many organizations such as ISPD have issued guidance on the application of NIPT in sex chromosome aneuploidy [18]. Fetal free DNA sequencing can be used to screen for sex chromosome abnormalities, but the detection rate and false positive rate are not as good as those of the three major chromosomes. Pregnant women should be informed that the positive result may be the mother's fetal sex chromosome abnormalities, and further invasive prenatal examination of maternal chromosomes is needed. However, pregnant women have the right to refuse to do fetal sex chromosome aneuploidy screening [17].

In this study, there were false-positive and false-negative results in single-twin pregnancies, three false-negative cases and 43 false-positive cases were screened out of 14574 singleton pregnant women; 14 false-positive cases were screened out of 6471 twin-born pregnant women. There are many reasons for NIPT false positive and false negative results: 1) Low fetal DNA concentration; 2) Maternal chromosomal abnormalities; 3) Restricted placental mosaicism; 4) Fetal mosaicism; 5) Disappearance of twins. In this study, the total cell-free DNA concentration of twin pregnancies was above the standard required by NIPT, in the massively parallel sequencing, the cell-free DNA concentration of individual fetus could not be measured separately. From the results of placental chromosome karyotype, abnormal maternal copy number and placental mosaicism are the main reasons for inconsistent results. Moreover, the NIPT data analysis method cannot clarify whether the abnormality is caused by maternal copy number abnormality or fetal mosaicism. Therefore, for NIPT copy number abnormalities or chimera results, maternal leukocytes, amniotic fluid cells, and multiple placental tissues should be collected for verification. In addition, the current NIPT based on SNP and targeted sequencing technology has not been routinely used for clinical twin pregnancy screening for aneuploidy.

The overall sensitivity of NIPT for the detection of the three major chromosomal aneuploidies of singletons is 96.67%, and the false positive rate is the highest 0.04%, which are consistent with other reports [3]. Risk factors for singleton detection in this study contain: Tang Si high risk and borderline risk, abnormal fetal ultrasound structure, abnormal fetal ultrasound soft index, and age ≥ 35 years due to delivery. According to statistical analysis, the positive rate of the three major chromosomal aneuploidies in pregnant women with high-risk factors is significantly higher than that of pregnant women without high-risk factors. More suspected cases can be found in the group of pregnant women with high-risk factors. Therefore, it is advisable that standard guideline for NIPT incorporating risk factors and clinical indications should be established.

In addition, in this study, NIPT also detected a total of six cases of other abnormalities in single and twin pregnancy, including copy number abnormalities, mother or fetus origin, etc., which have also been reported in previous studies [19]. Therefore, non-invasive prenatal screening cannot be used as a prenatal diagnosis method. This point should be explained when genetic counseling is given to patients.

At present, NIPT as a prenatal screening technology has been widely used in singleton pregnant women in China. Our research confirms that in twin pregnancy pregnant women, NIPT can still achieve similar detection efficacy and the performance seems to be better than traditional screening. The number of cases of sex chromosome aneuploidy in this study is too low to accurately assess the screening efficiency. However, NIPT can effectively detect aneuploidy of the three major chromosomes and can be included in the current screening system under the premise of strict control of laboratory technical procedures.

Conflict of interest

All authors declare no conflict of interest.

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Table 1. Non-invasive prenatal screening data of 6471 pregnant women with twin pregnancy

Chromosome Abnormality	Ture positive	True negative	False negative	False positive	Sensitivity	Specificity	Rate of false negative	Rate of false positive	PPV*	NPV*	Yorden Index
T21	18	6451	0	2	100.00	99.97	0.00	0.03	90.00	100.00	99.97
T18	6	6462	0	3	100.00	99.95	0.00	0.05	66.67	100.00	99.95
T13	1	6468	0	2	100.00	99.97	0.00	0.03	33.33	100.00	99.97
SCAs	39	6426	0	6	100.00	99.91	0.00	0.09	86.67	100.00	99.91
Abnormality-	4	6466	0	1	100.00	99.98	0.00	0.02	80.00	100.00	99.98

other chromosomes											
Sum	68	6389	0	14	100.00	99.78	0.00	0.22	82.93	100.00	99.78

*PPV — positive predictive value; *NPV — negative predictive value

Table 2. Non-invasive prenatal screening data of 14,574 singleton pregnant women in Southern Hospital from 2015 to June 2017 **(there is no link in the text???)**

Chromosome Abnormality	Ture positive	True negative	False negative	False positive	Sensitivity	Specificity	Rate of false negative	Rate of false positive	PPV*	NPV*	Yorden Index
T21	45	14525	1	3	97.83	99.98	2.17	0.02	93.75	99.99	97.81
T18	9	14562	0	3	100.00	99.98	0.00	0.02	75.00	100.00	99.98
T13	4	14569	1	0	80.00	100.00	20.00	0.00	100.00	99.99	80.00
Sum of above three	58	14508	2	6	96.67	99.96	3.33	0.04	90.63	99.99	96.63
Sex chromosomes	17	14532	1	24	94.44	99.84	5.56	0.16	41.46	99.99	94.28
Others	2	14559	0	13	100.00	99.91	0.00	0.09	13.33	100.00	99.91

*PPV — positive predictive value; *NPV — negative predictive value

Table 3. Comparison of detection rate of abnormality by NIPT in singleton pregnant women with high and low risk group **(there is no link in the text???)**

Risk group	High risk （case）	True Positive (cases)	Abnormality detection rate （%）
High-risk	94	50	53.19
Low-risk	17	8	47.05
Sum	111	58	52.25

Statistics — $x^2 = 5.441$; p = 0.020