CEOG Clinical and Experimental Obstetrics & Gynecology

Non-invasive prenatal screening for chromosome 21, 18, and 13 aneuploidies in a mixed risk factors pregnancy population

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Summary

Non-invasive prenatal screening (NIPS) using cell-free DNA is being offered to an increasing number of pregnant women. In this observational study, the authors found chromosome 21, 18, and 13 aneuploidies in a mixed risk factors pregnancy population using NIPS, and found 23 cases with NIPS high risk. They also found that the NIPS results of two cases with hysteromyoma were positive. There were 61 cases in all with maternal gynaecological tumor and the positive rate was 3.28%, which suggests that there may be a specific relationship between them and more evidence is urgently required. Among 23 cases, most had normal results with early or middle pregnancy serologic examination or ultrasonic testing. Therefore, NIPS is more accuracy and could mostly make up for regular prenatal in diagnosis. In addition, NIPS could avoid the risks such as intrauterine infection and miscarriage caused by amniocentesis karyotyping analysis, therefore, the clinical promoter of NIPS during pregnancy is very meaningful.

Key words: Fetal aneuploidies; Non-invasive prenatal screening (NIPS); Mixed risk factors; Pregnant women.

Introduction

Fetal aneuploidies, such as trisomy 21 (T21, Down syndrome), trisomy 18 (T18, Edwards syndrome), and trisomy 13 (T13, Patau syndrome) are the most common chromosomal abnormalities, as well as the sexual chromosomes (X and Y). In the recent ten years, the traditional prenatal aneuploidy screening methods had been violently challenged and reformed in the world. At the moment, the combined prenatal test, including different maternal serum biochemical screening tests offered in the first or second trimester, combined with nuchal translucency (NT) measurement were able to identify 75-85% aneuploidies. Following that, the invasive diagnostic testing was considered in the high-risk population [1]. Invasive prenatal diagnosis, amniocentesis or chorionic villus sampling was taken for fetal karyotyping or rapid aneuploidy detection [2, 3]. Non-invasive prenatal screening (NIPS) for fetal aneuploidy with the use of cell-free DNA (cfDNA) obtained from maternal plasma was introduced into clinical practice from 2011. Such screening has been reported to have a detection rate for T21 ≥ 99%, with a false positive rate ≤ 0.1% [4]. The high accuracy of NIPS has also rapidly expanded to include other common aneuploidies for T13, T18, X, and Y. Nevertheless, the study in groups with mixed risk factors has not yet been well processed. This observational study aimed to report the performance of non-invasive prenatal aneuploidy screening in mixed risk factors of pregnancy population and discovered that the clinical promotion of NIPS during the early and middle pregnancy is necessary. Moreover, the chromosome karyotyping analysis or ultrasound examination is necessary in those pregnant women with NIPS examination which could reduce the false positives and negatives of NIPS and confirm the fetal condition.

Materials and Methods

From October 2014 to December 2015, 1031 pregnancies asked for NIPS test to avoiding fetal aneuploidies. The qualification of laboratory undergoing NIPS was certified by national commission. The exclusion criteria for pregnant women were as follow: (1) women with multiple pregnancies, (2) women with confirmed chromosomal aneuploidies or other chromosomal abnormalities, and (3) women with gestational age above 23 accepting amniocentesis. With the primary pre-test counseling, 24 cases were excluded (18 cases were multiple pregnancies, six cases were confirmed chromosomal abnormal carriers). Informed written consent was obtained from all pregnant women who agreed to take NIPS test.

The authors sampled the maternal blood and observed the data collected from the remaining pregnant women undergoing NIPS. The NIPS method was performed by semiconductor sequencing [5]. The data included maternal weight, maternal anxiety, maternal age (MA), body mass index (BMI), and gestational age (GA) (Table 1). Risk factors included advanced maternal age (≥ 35-

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Revised manuscript accepted for publication November 14, 2016

Table 1. — *Maternal characteristics and gestational age of blood sampling.*

Maternal age (years old)	Number	Percentage (%)
≤25	63	6.27
26-30	306	30.44
31-34	209	20.80
35-38	295	29.35
39-42	109	10.85
≥43	23	2.29
Median(years old)	33	
Advanced maternal age (≥35 years old)	427	42.49
Gestational age at blood sampling (weeks)		
6-8	1	0.10
9-12	17	1.69
13-16	204	20.30
17-20	697	69.35
21-24	76	7.56
25-28	9	0.10
≥29	1	0.09
Range (weeks)	7-32	
Median (weeks)	18	

Table 2. — *Methods of attaining pregnancies.*

Method of conception	Number of pregnancies	Percentage (%)
IVE	42	4.18
ICSI	9	0.90
Nature	951	94.63
Unknown	3	0.30
Total	1005	100.00

years-old), history of previous miscarriage, abnormal NT, risk of maternal serum screening (three markers), and risk result from second trimester screening. Patients receiving artificial reproductive technology (ART) to conceive (Table 2), and with maternal gynecological or other tumors, HBV or HCV carriers or infectors were also enrolled for analysis (Table 3).

The NIPS test results were compared to those obtained by aneuploidy fluorescence in situ hybridization, chromosome karyotyping analysis, and/or microarray. Chromosome karyotyping analysis was performed on amniotic fluid and/or umbilical cord blood. Specific experimental processes are seen in Figure 1.

Results

Of the 1,007 pregnancies, the NIPS testing results from 1,005 pregnant women in center for laboratory diagnosis, Yantai Yuhuangding Hospital affiliated to Medical College of Qingdao University, were analyzed in this study. Maternal age ranged from 22 to 45 years (Table 1), and the median was 33 years. The group aged 26-30 years was the majority (306, 30.44%) in the entire group. The pregnant women more than 35 years old were 42.49%. Gestational age at blood sampling ranged from seven to 32 weeks, and

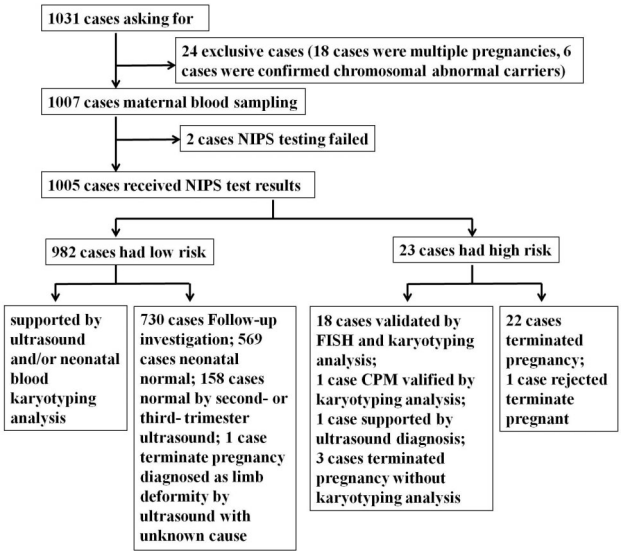

Figure 1. — The flow chat of the study.

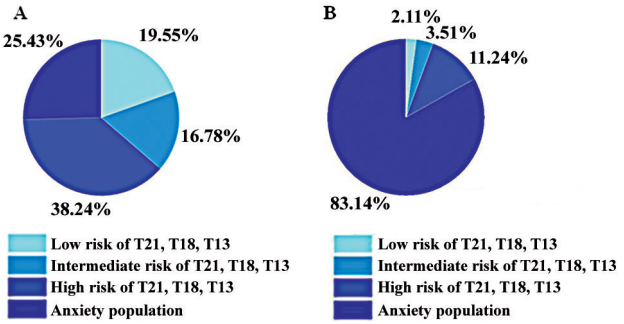

Figure 2. — Indication results of serologic test for patients. (A) Results for patients less than 34-years-old. (B) Results for patients more than 35-years-old.

Table 3. — *Special cases asking for NIPS.*

Situation of other population for NIPS	Number (percentage [%])
Abnormal pregnancy history	129 (12.84)
HBV* carriers or infectors	9 (0.90)
HCV** carriers	1 (0.10)
Maternal gynecological tumor	61 (6.07)
Other tumors	6 (0.60)
Others	6 (0.60)

** Hepatitis B virus. ** Hepatitis C virus.*

the median was 18 weeks; 69.35% of the group had a gestational age from 17 to 20 weeks. The information of BMI included 977 pregnant women, ranging from 17.4 to 47.7. Thirty-four pregnancies had a BMI more than 32.0.

During the study, 0.90% (9/1005) pregnancies needed to resample result from NIPS because of failure in quality control criteria mostly because of early gestational age. The

Table 4. — *Information of pregnancies with high risk of NIPS.*

No.	NIPS	Karyotyping analysis	MA	GA	BMI	Abnormal pregnancy history	Ultrasound test	Serum screening T21	Serum screening T18
1	T21	47,XX,+21	38	21	None	None	None	None	None
2	T21	47,XY,+21	33	18	24.4	None	None	None	None
3	T21	47,XX,+21	31	17	24.8	None	None	1/130	None
4	T21	47,xx,+21[20]/47,xx,+der(21;21)(q21;q21)[30]/46,XN[112]	32	20	23.3	Spontaneous abortion	None	1/76	None
5	T21	47,XX,+21	29	19	27.0	Threatened abortion	None	1/843	None
6	T21	47,XX,+21	27	18	21.6	Threatened abortion	None	1/60	None
7	T21	47,XY,+21	32	19	22.2	None	None	1/77	None
8	T21	47,XX,+21	33	18	27.1	Embryo damage	None	1/286	None
9	T21	45,XX,der(14;21)(q10;q10)	30	12	23.0	Robertsonian translocation	NT=4.7mm	None	None
10	T21	47,XX,+21	34	18	25.3	None	None	1/56	None
11	T21	47,XX,+21	32	20	25.4	None	None	1/300	None
12	T21	47,XX,+21	36	18	23	Spontaneous abortion	None	1/329	None
13	T21	47,XX,+21	28	12	19.8	Embryo damage	NT=4.2mm	None	None
14	T21	47,XX,+21	31	18	21.5	None	NT=2.2mm	1/247	None
15	T21	47,XX,+21	27	13	18.4	None	NT=3.3mm	None	None
16	T21	47,XX,+21	31	18	21.5	None	NT=2.2mm	1/247	None
17	T21	47,XX,+21	27	13	18.4	None	NT=3.3mm	None	None
18	T18	47,XX,+18	42	17	27.5	None	None	None	None
19	T18	None	43	15	32.0	None	Fetal forearm malformation	None	None
20	T18	None	43	15	27.7	Embryo damage	NT=7.9mm	None	None
21	T X	47,XXX	40	17	21.3	None	None	None	None
22	MX	45,XO[70]/46,XX[30]	29	17	27.9	None	None	1/566	1/976
23	XXY	47,XXY	29	17	22.0	Threatened abortion	None	1/65	None

test for these cases usually took another seven to 12 working days from resampling to report delivery. Additionally, the NIPS testing of one case failed to result after resampling.

For pregnant women less than 34 years old (578 cases), 147 cases (147/578, 25.43%) with normal results of ultrasound and no maternal serum screening asked for NIPS due to purely anxious feelings of possible fetal aneuploidies ans 113 pregnant women (113/578, 19.55%) asking for NIPS were at low risk for T21, T18 or T13. Other indications included high risk for T21, T18 or T13, which accounted for 221 pregnant women (221/578, 38.24%), and intermediate risk for T21, T18 or T13, which accounted for 97 (97/578, 16.78%) (Figure 2A). For pregnant women with an age greater than 35 years (427 cases), pure anxiety about amniocentesis was the most common reason for the pregnant women selecting NIPS for fetal aneuploidy screening, of which 355 pregnant women (355/427, 83.14%) chose NIPS with normal results of ultrasound and no maternal serum screening. Nine pregnant women asking for NIPS was at low risk for T21, T18 or T13. Other indications included 48 (48/427, 11.24%) pregnant women at high risk for T21, T18 or T13 and 15 (15/427, 3.51%) pregnant women at intermediate risk for T21, T18 or T13 (Figure 2B).

Of 1,005 cases, 129 cases (129/1005, 12.83%) had abnormal pregnancy history. Eleven cases had the history of spontaneous abortion. Sixty-nine cases had threatened abortion; among these, nine cases had embryo damage as well, one case also had HBV infector, one case had fetal ascites, and one case once had T21 fetal and terminated pregnancy one year ago as well. Forty-four cases had a history of embryo damage at less than 16 weeks of gestation; among these, nine cases were at risk as well, and one case also had Robertsonian translocation. In addition to these, there were 15 cases with abnormal fetus, including hydatid pregnancy, ventricular septal defect, ectopic pregnancy, fetal lymphangioma, fetal with cerebral palsy, fetal progressive muscular dystrophy, fetal meconium peritonitis, anencephalus, and hereditary hearing loss carrier. The authors also analyzed other population with disease in pregnant women asking for NIPS test (Table 3), and found that women with HBV carrier or infectors were 9(0.90%); HCV carrier was one (0.10%) and with maternal gynecological tumor were 61 (6.07%); among these, 31 cases had uterine fibroid. Other tumors were six (0.60%) and other special situations were six (0.60%). Six cases of other tumors included two cases of thyroid adenoma, two cases with both chondroma and embryo damage, one case with thyroid papillary tumor, and one case of nasopharynx cancer and hypothyroidism as well.

The NIPS test result of ≥ 3 is defined as high risk, and < 3 as low risk. Among 1,005 pregnant women, 23 cases (23/1005, 2.29%) had high risk results from NIPS analysis (Table 4). Seventeen cases were at high risk for T21 (positivity rate 17/1005, 1.69%) and three cases were at high risk for T18 (positivity rate 3/1005, 0.30%). Of the positive cases, four cases were found to be normal by routine first-trimester ultrasonographic NT measurement and three cases were abnormal with NT value 4.2 mm, 4.7 mm, and 7.9 mm, respectively. Six cases were at high risk for serum T21, T18 or T13 screening, and five cases were at intermediate risk for serum screening. Notably, twio positive cases were low risk for serum T21, T18 or T13 screening. Among 23 positive cases, 18 cases were supported by the later aneuploidy fluorescence in situ hybridization (FISH) and chromosome karyotyping analysis sampling with amniotic fluid and/or neonatal blood; one case had CPM vilified by karyotyping analysis, one case supported by ultrasound diagnosis, and three cases terminated pregnancy without karyotyping analysis. After diagnosis, 22 pregnant women decided to terminate pregnancy and one case rejected terminating pregnant.

Among 23 positive cases, one case of 14 and 21 chromosome Robertsonian translocation (45, XX, der(14;21)(q10;q10)) (No.9 in Table 4 (1)), ultrasonographic result showed that NT value was 4.7 mm, and then the pregnant woman chose NIPS due to worries about invasive procedures that may cause risks such as miscarriage. The NIPS test result displayed a high risk of T21, and then she accepted and chose to terminate pregnancy without fetal karyotyping analysis. Another case was 45, XO (No.22 in Table 4 (2)) verified by the result of the NIPS, and then also confirmed as 45, XO [70]/46, XX [30] with further amniocentesis karyotyping analysis, deciding to terminate pregnancy without puncture of the umbilical cord blood karyotyping analysis. One case of T21 at high risk (No.4 in Table 4 (1)) was diagnosed by chromosome karyotyping analysis as confined placental mosaicism (CPM) (47, XX, +21[20]/47, XX, +der(21;21)(q21;q21) [30]/46, XN[112]). The percentage of chimeric cells was less 10%, and the following umbilical cord blood karyotyping analysis result showed that the fetus was normal, and that the pregnancy was undergoing. Now the newborn at one month, has no obvious phenotypic abnormalities, and the mental development remains unknown.

The sensitivity for fetal T21, T18 or T13 aneuploidy screening in this study was 100% (23/23). The specificity for fetal T21, T18 or T13 aneuploidy screening in this study was 99.90% (1,004/1,005).

Discussion

Since the presence of fetal DNA fragments in maternal plasma was discovered [6], the non-invasive technology based on fetal DNA fragments sequencing for prenatal aneuploidy screening has emerged. In United States, non-invasive prenatal screening had come into clinical practice since 2011. Professional societies [7-11], including the American College of Obstetricians and Gynecologists (ACOG), the Society for Maternal-Fetal Medicine (SMFM), National Society of Genetic Counselors (NSGC), Society of Obstetricians and Gynaecologists of Canada (SOGC), American College of Medical Genetics and Genomics (ACMG), Japan Society of Obstetrics and Gynecology (JSOG), and Italian College of Fetal Maternal Medicine *et al.*, had currently recommend offering NIPS test as a primary screening to pregnant women at high risk for fetal aneuploidy, reserving invasive diagnostic procedures for those at the highest risk. Additionally, the importance of genetic counseling (pre- and after- NIPS) and informed consent principle were also addressed by the Societies.

Amniocentesis karyotyping analysis is a mean of screening abnormal fetus for pregnant women have serum screening at high risk or abnormal ultrasonographic result, but it could cause other risks such as intrauterine infection and miscarriage. As women of advanced age find it more difficult to become pregnant, they are more concerned with the potential risk of invasive procedures. Therefore, fetal T21, T18 or T13 screening through peripheral blood of pregnant women is the right choice for pregnant women at 12 to 24 weeks of pregnancy. The index included in the NIPS test of this study was as follows: (1) positive conventional serum Down syndrome screening tests in 426 cases, (2) abnormal sonographic findings in 16 cases, (3) advanced maternal age (≥ 35-years-old) in 427 cases, and (4) other reasons such as maternal anxiety in 502 cases.

NIPS is only recommended for patients in the high risk group [9]. Its application in the low risk group is still a matter of conflict. A study published in 2014 reported that 1914 women at general risk in the obstetric population showed significantly lower false positive rates and higher positive predictive values for detection of T21 and T18 when the physicians applied NIPS rather than traditional screening [12]. In this study, 23 pregnancies were NIPS at high risk; of these, four pregnancies were normal with ultrasound test result (Table 4). In this case, traditional ultrasound test could not meet the demand of accuracy, and amniocentesis karyotyping analysis has known risks, hence, a simple, safe, and accurate means needs to be added to the prenatal ordinary detection. For fetal maternal specialists, how to apply NIPS and which group of patients should be offered NIPS requires more evidence from further clinical samples.

Many NIPS studies also found the phenomenon of the chromosome of micro repeat or miss, which could cause the false positive cases with only serological screening in Downs syndrome [13]. The presence of chromosomal variants, as well as deletions, duplications, and other rare anomalies, did not interfere with detection of T21 or T18, but the tracking observation for these pregnant women and

fetuses was still an important part in the study of NIPS result tracking. In the present study, the ultrasonic examination of two chromosome micro-repeated cases of the chromosomes 2 and 4 repeats was normal. Then maternal blood karyotyping analysis confirmed respectively of the maternal chromosomes 2 and 4 repeats, resulting in the interference phenomenon caused by maternal chromosomes, and normal fetal phenotypic after birth. In some cases chromosomes 1, 12, 13, and 8 were found and in the remaining of the chromosomes 2 and 4 micro-repetitions and/or absence of micro were found. After tracking observation, no obvious abnormity under ultrasound was seen. The pregnancy still continued and had normal fetus within half a year of birth. Also, the present authors found one case with a NIPS result of 47, XXY, and the serological screening for Down syndrome showed a high risk. Then this pregnant woman accepted amniocentesis karyotyping analysis, and the result showed no abnormal chromosome 21, which was 47, XXY, which further illustrated the accuracy is higher in NIPS than the current prevailing early pregnancy check and the middle stage pregnancy serological screening for Down syndrome.

The present results showed that the clinical promotion of NIPS check could greatly make up for regular prenatal (early and middle pregnancy serologic test and ultrasonic examination) in diagnosis. Considering the serum low risk pregnant women and those less than 34 years of age pregnant women included in the checked range of NIPS, both reduced the risk of amniocentesis and compensated for early pregnant screening misdiagnosis because of false negatives. It is also very meaningful for NIPS in serum low risk population. Currently, more and more pregnant women have a positive attitude towards NIPS test and would like to use the test if available according to Swedish cohort of 1,003 pregnant women test [14]. The present authors believe that more and more Chinese pregnant women would accept this test with its development as well.

In this study, the authors found one case with false positive NIPS result; the specific situation was as follows: the T21 high risk pregnant woman was determined as the confined placental mosaicism (CPM) after FISH to the placenta and amniotic fluid puncture karyotyping analysis, and the percentage of chimeric cells was less 10%. The following umbilical cord blood karyotyping analysis result showed that the fetal was normal, which indicated that the NIPS result was false positive. Many NIPS studies showed that placental chimeric of trisomy is the main factor of NIPS results in false positives [13, 15]. Therefore, the chromosome karyotyping analysis is necessary for those pregnant women with positive NIPS result or at high risk [16, 17].

There is much research assessing the relationship between maternal tumor and NIPS detection. As reported before, Prasad *et al.* [18] found that the routine use of NIPT led to the discovery of maternal neoplasms. Amant *et al.* [19] also showed that NIPS may enable accurate presymptomatic detection of maternal tumors and treatment during pregnancy with NIPS test in over 4,000 prospective pregnancies. Of the 23 cases in this present study, two cases had hysteromyoma, one case (No.21) which was 40-years-old, the BMI value was 21.3 and the tumor size was 7.6×5.4 cm^2. The following umbilical cord blood karyotyping analysis result showed as 47, X trisomy. The other case (No.12) was 36-years-old and the BMI value was 23, with one miscarriage and a threatened abortion at this time of pregnancy at eight weeks. The fetus was at T21 high risk with karyotyping analysis. Hence there existed a relationship between maternal gynecological tumor and NIPS test, and whether maternal gynecological tumor could contribute to positive value for NIPS result requires more evidence.

On the whole, NIPS is more accurate than traditional detection and could greatly make up for regular prenatal (early and middle pregnancy serologic test and ultrasonic examination) in diagnosis, and it is also very meaningful in traditional detection, such as amniocentesis karyotyping analysis of screening abnormal fetus for pregnant women which could cause intrauterine infection, miscarriage, and other risks, especially for those with advanced maternal age (≥ 35-years). Therefore, it is necessary for a clinical promotion of NIPS during early and middle pregnancy. Moreover, the chromosome karyotyping analysis is important for those women with placental chimeric of trisomy. Those could reduce the false positive of NIPS and confirm the fetus' condition.

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