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Outcomes of 8 Years of Noninvasive Prenatal Testing at Nippon Medical School Hospital

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Background: Noninvasive prenatal testing (NIPT) is used to screen for fetal chromosomal abnormalities, such as fetal aneuploidy, and has been offered at our hospital since 2013. We analyzed data from our center to determine if NIPT screenees could be given more-accurate information on NIPT outcomes.

Methods: This retrospective observational study included 819 pregnant women who requested NIPT at Nippon Medical School Hospital from November 2013 to October 2021. We examined medical records for data on NIPT results and clinical outcomes.

Results: Of the 819 women, 764 (93.2%) underwent NIPT, and 55 (6.7%) did not. Of the 764 women who underwent NIPT, 17 received a positive result (2.2%), of whom 2 (11.8%), 4 (23.5%), and 11 (64.7%) received a positive result for trisomy 13, 18, and 21, respectively. The true-positive rates after definitive diagnoses of trisomy 13, 18, and 21 were 1 (50%), 3 (75%), and 11 (100%), respectively. Of the 17 positive results, there were two false-positive results (11.8%) (for trisomy 13 and trisomy 18). Eleven women with fetal aneuploidy terminated their pregnancies, and four cases resulted in intrauterine fetal death. Five neonates with negative NIPT results had congenital disease without chromosomal abnormality. Two patients had indeterminate results from the first blood sampling, possibly because of treatment with unfractionated heparin. The results of repeat testing after heparin cessation were negative.

Conclusions: Our results were generally similar to nationwide data for Japan. NIPT providers can provide more detailed and individualized genetic counseling for each situation by understanding their own medical facility's data in detail. (J Nippon Med Sch 2022; 89: 520–525)

Key words: noninvasive prenatal testing (NIPT), prenatal test, genetic counseling

Introduction

Fetal chromosomal abnormalities are diagnosed by using amniocentesis and chorionic villus sampling; however, these tests slightly increase the risk of miscarriage. Before invasive tests are ordered, several medical centers in Japan perform prenatal screening of fetal chromosomal abnormalities such as maternal serum screening (including α-fetoprotein, human chorionic gonadotropin, unconjugated estriol, inhibin A), first-trimester combined testing (including maternal age, fetal translucency, fetal heart rate, and serum free β-human chorionic gonadotropin), and noninvasive prenatal testing (NIPT).

Before 2013, our hospital provided only amniocentesis for diagnosing prenatal chromosomal abnormalities. However, to avoid miscarriage by invasive amniocentesis, NIPT was introduced in November 2013. Many pregnant women who visit our hospital are older (>35 years) or have a history of repeated pregnancy loss and so are concerned about potential chromosomal abnormalities. For these women, genetic counseling is provided by a specialist obstetrician, clinical geneticist, or certified genetic counselor. We attempt to address their concerns

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during the counseling and proposed NIPT, amniotic fluid test, and/or ultrasound examination, when necessary. Many pregnant women choose NIPT after genetic counseling.

NIPT identifies underlying genetic pathologies of fetal aneuploidy by analyzing fetal genetic material and cell-free fetal DNA (cfDNA) in maternal circulation. Mandel et al.[1] first reported cfDNA in plasma in 1948, and Dennis Lo et al.[2] reported the presence of cfDNA in the plasma of pregnant women. In a pregnant woman, cfDNA is derived from the turnover of maternal cells and fetal cytotrophoblasts of the chorionic villi. With the advent of massively parallel sequencing, NIPT is now technically feasible for all pregnancies. NIPT has a high detection rate and can determine the fetal risk of genetic disorders without the risk of miscarriage.

Beginning in 2013, NIPT was offered in the United States, Europe, and China and is currently performed in more than 90 countries worldwide. NIPT has been performed in clinical research at only a limited number of centers that have a genetic counseling system in Japan, and provider institutions have been certified by the Japanese Association of Medical Sciences since April 2013[3]. NIPT for fetal aneuploidy detection has been offered since November 2013 at our hospital, and all women considering prenatal tests can receive genetic counseling. The Japan NIPT Consortium, the largest research group in Japan, has been accumulating data on pregnant women who underwent NIPT, as part of a multicenter observational study and report[3]. However, the overall data do not allow for interpretation of detailed individual information, despite the need for individualized support in genetic counseling.

In this study, we described the test results, number of indeterminate results, and role of genetic counseling in NIPT over an 8-year period at Nippon Medical School Hospital. After analyzing data from our center, we examined whether applicants for NIPT could be given more-accurate information regarding NIPT outcomes.

Materials and Methods

We retrospectively analyzed medical data for all pregnant women who underwent NIPT at Nippon Medical School Hospital between November 2013 and October 2021. The criteria for accepting an NIPT application were age 35 years or older at the time of delivery or positive findings of fetal abnormality on sonographic screening, such as nuchal translucency thickness; a gestational age from 8 weeks 0 days to 13 weeks 6 days; and intention to deliver their child at our hospital. In cases of pregnancy assisted by reproductive technology, the minimum maternal age when the patient underwent ovum collection was 34 years and 2 months. Pregnant women and their partners who were considering NIPT received mandatory genetic counseling.

Approximately 20 mL of peripheral blood was collected after obtaining informed consent for participation in this study. Blood samples were sent to GeneTech Inc. (Tokyo, Japan) from 2011 to 2015 and again starting in 2021. From 2016 to 2020, blood samples were sent to Eurofins Genomics Inc. (Tokyo Japan). Sample analysis was performed by using massively parallel sequencing to detect fetal aneuploidy (trisomy 21, 18, and 13). Within 2 weeks of blood sampling, we disclosed the results to couples during genetic counseling. When the result of NIPT was positive, we provided additional detailed clinical information through fetal examination, using ultrasonography and amniocentesis for definite diagnosis. To reach a meaningful decision, we conducted several genetic counseling sessions with couples before and after definite fetal diagnoses.

The pregnancy outcomes of interest, which were obtained from medical records and medical referral letters, were spontaneous pregnancy loss, elective termination, live birth, and gestational age at the time of these outcomes. We obtained consent for this study from all pregnant women and their partners when they received genetic counseling. This retrospective study was approved by the Institutional Research Ethics Board at Nippon Medical School (B-2019-024).

Results

During the study period, there were 3,656 births; 819 women, including five with twin pregnancies, underwent genetic counseling for NIPT. Of these 819 women, 764 (93.2%) underwent NIPT, and 55 (6.7%) did not, for various reasons.

Outcomes of pregnant women who did not undergo NIPT

A total of 55 women declined NIPT: 38 made the decision after, and 17 before, genetic counseling. Among the women who did not receive NIPT after genetic counseling, 13 selected amniocentesis without NIPT because most had a history of spontaneous recurrent pregnancy loss and requested information on general fetal chromosomal abnormalities, not only for trisomy 13, 18, and 21. Twenty-two women received no prenatal genetic testing. Most women were initially hesitant to receive NIPT and

Table 1 Outcomes of Positive NIPT Cases

case	maternal age (years)	reason for taking NIPT	NIPT result	definitive diagnosis	methods of definitive diagnosis	outcomes
1	37	advanced age	T13	normal karyotype	AC	normal neonate
2	39	history of termination of T21 pregnancy	T13	T13	AC	TOP
3	35	advanced age	T18	T18	AC	IUFD (17w)
4	37	advanced age	T18	T18	villi sampling	IUFD (14w)
5	40	advanced age	T18	normal karyotype	AC	normal neonate
6	41	hydrops fetalis	T18	T18	villi sampling	IUFD (15w)
7	33	NT thickness	T21	T21	AC	TOP
8	35	advanced age	T21	T21	AC	TOP
9	35	advanced age	T21	T21	AC	TOP
10	36	advanced age	T21	T21	AC	TOP
11	37	advanced age	T21	T21	AC	TOP
12	39	advanced age	T21	T21	AC	TOP
13	42	advanced age	T21	T21	AC	TOP
14	42	advanced age	T21	T21	AC	TOP
15	45	Previous child with cerebral palsy	T21	T21	AC	TOP
16	44	NT thickness	T21	T21	villi sampling	IUFD (13w)
17	46	NT thickness	T21	T21	AC	TOP

NIPT, noninvasive prenatal genetic test. T13, trisomy 13. T18, trisomy 18. T21, trisomy 21. NT, nuchal translucency. AC, amniocentesis. TOP, termination of pregnancy. IUFD, intrauterine fetal death

decided not to terminate the pregnancy regardless of fetal condition after genetic counseling. Of the 17 women who canceled NIPT before genetic counseling, one had a miscarriage and another was diagnosed as having an anencephalic fetus before her counseling session.

Outcomes of pregnant women who underwent NIPT

Seventeen (2.2%) of 764 patients who received NIPT had positive test results (**Table 1**). The breakdown of the positive results was 2 (11.8%), 4 (23.5%), and 11 (64.7%) diagnoses of trisomy 13, 18, and 21, respectively. Amniocentesis was recommended in all cases, for confirmation of diagnosis with additional genetic counseling. Intrauterine fetal death occurred before amniocentesis in three patients (two with trisomy 18 and one with trisomy 21). We obtained definitive results from the villi of the stillbirths. The remaining 14 patients underwent amniocentesis at our hospital. One case of trisomy 18 resulted in fetal death after amniocentesis. Analysis of positive NIPT results yielded a positive predictive value for NIPT, as confirmed by amniocentesis or villi sampling of the stillbirth, of 1 (50%), 3 (75%), and 11 (100%) for trisomy 13, 18, and 21, respectively. One case each of trisomy 13 and trisomy 18 determined by NIPT had a normal karyotype on amniocentesis and proved to be a false-positive for NIPT. All women diagnosed with fetal aneuploidy by amniocentesis during fetal life chose to terminate their pregnancy. We provided genetic counseling with a detailed fetal examination using ultrasound after explaining the positive results. Most women requested consultation with a genetic counselor and a maternal-fetal medicine specialist, but a few women did not. One patient requested a change in hospital for amniocentesis and painless delivery if she were to select pregnancy termination after obtaining a confirmed diagnosis of aneuploidy. Trisomy 21 was diagnosed by amniocentesis at a different hospital.

Outcomes of women with negative NIPT results

We reviewed neonatal outcomes after birth in 711 of the 747 women who had negative NIPT results. We identified five cases (0.7%) of congenital disease among these neonates, namely, congenital heart disease (double outlet right ventricle), diaphragmatic hernia, esophageal atresia, cleft lip and palate, and multiple malformations. The infant with multiple malformations had cleft lip and palate, microphthalmia, nasal agenesia, and deafness. In another case, a woman lost her fetus in the third trimester from an unknown cause.

Indeterminate results for NIPT

During the study period, NIPT results were indeterminate in two cases. Both patients were treated with unfractionated heparin for recurrent pregnancy loss. In addition, their results were obtained after the change in test-

ing company, in 2021. We instructed them to temporarily stop heparin administration on the day of the retest. The results of the retest were negative.

Discussion

This study presents the results of NIPT over an 8-year period at our hospital. A total of 764 women received NIPT. Positive results were observed in 17 patients (2.2%); 15 diagnoses (88.2%) were confirmed as aneuploidy by amniocentesis or villi sampling, and two diagnoses (11.8%) were false positives. Intrauterine fetal death occurred in four cases, and 11 women selected termination of pregnancy after genetic counseling. Of the 711 women who had negative NIPT results and gave birth, five (0.84%) children had congenital diseases not involving a chromosomal disease. Two patients who were treated with unfractionated heparin had indeterminate results.

NIPT has a very high sensitivity and specificity for trisomy 13/18/21. In a meta-analysis, Mackie et al.[4] confirmed the accuracy of NIPT: sensitivity was 90.6%, 97.7%, and 99.4% for trisomy 13, 18, and 21, respectively. Gil et al.[5] reported that analysis of NIPT could detect more than 99% of trisomy 21 cases, 98% of trisomy 18 cases, and 99% of trisomy 13 cases; the false-positive rate was 0.13%. Because of its accuracy, NIPT is used to screen for fetal aneuploidy in many countries. In Japan, data have been collected by the Japanese Association of Medical Sciences since its introduction in 2013. Samura et al.[3] presented data on a total of 30,613 NIPT tests performed for Japanese women at 55 centers from 2013 to 2016. They reported an NIPT positive rate of 1.81%. The positive predictive values were 63.6%, 82.8%, and 96.5% for trisomy 13, 18, and 21, respectively. The false-positive rate was 8.8%. In data from other countries, Norton et al.[6] reported that the positive predictive value of NIPT was 80.9% for trisomy 21. Porreco et al.[7] analyzed 3,430 women at high risk and reported positive predictive values of 100% for trisomy 13 and 18 and 97.9% for trisomy 21. In the present study, the positive result for NIPT was 2.2%, and the positive predictive values were 50%, 75%, and 100% for trisomy 13, 18, and 21, respectively. Our false-positive rate was 11.8% (2 of 17 positive cases). It is difficult to compare these studies because the number of cases and participant characteristics differed greatly. However, all studies have reported excellent test performance. In addition, we reported fetal outcomes after a negative NIPT result and reasons for canceling NIPT after genetic counseling in our study. We believe that we are able to present these detailed results because the data were collected at a single center.

Only a limited number of facilities provide NIPT for pregnant women in Japan[3]. NIPT is conducted in compliance with guidelines on new prenatal genetic testing using maternal blood, which permit NIPT on the condition that a standard level of genetic counseling is provided. At our hospital, couples receive genetic counseling from an obstetrics and genetic specialist, and genetic counseling is provided before NIPT. Some women became more nervous after receiving NIPT. Suzumori et al.[8] found that women who underwent NIPT tended to have a high rate of depression and anxiety. Therefore, our hospital provides personalized support and management for each pregnant woman's situation, in collaboration with obstetrics and genetic counseling. After NIPT testing, a detailed ultrasound examination was performed in the early second trimester. Genetic counseling is not easy because of the need for individualized support for each unique situation and obstetrical condition. We ensured that explanations were provided during follow-up visits and that genetic counseling was provided by a specialist who discussed test results, strategy, and timing after or before genetic testing. We have developed a consulting system that provides psychiatrists and psychological counselors for women who develop severe mental health problems. At our hospital, the pregnant woman and her partner receive genetic counseling and hear the results together, so that pregnant woman receive the necessary support. In addition, multiple obstetrics and genetic examinations are provided, if requested. No woman reported depressive symptoms during the study period, which suggests our framework is effective.

A previous Japanese nationwide study[3] did not provide detailed information on women who received NIPT. Two patients (0.26%) had indeterminate results after the initial blood sampling; both had received unfractionated heparin in our study. A previous study[9] reported the maternal and other characteristics of women with indeterminate NIPT results: 0.32% of women had indeterminate NIPT results after initial blood sampling because of a low fetal fraction (<4%), maternal malignancy, and heparin administration in 20.0%, 16.4%, and 11.8% of cases, respectively. In addition, the risk of an indeterminate result was significantly higher for women receiving heparin than for those who were not. Gromminger et al.[10] reported that injection of low-molecular-weight heparin increased the number of short fragments of DNA with higher guanine and cytosine (GC) content, which affecting NIPT results.

It remains unclear whether smaller and GC-rich fragments are of fetal or maternal origin because there is no obvious systematic difference in the fetal fraction before and after heparin injection, and because the underlying cause of the described maternal injection of heparin effect is not yet completely understood. Although further study is needed, we believe that unfractionated heparin, and low-molecular-weight heparin, had an effect on indeterminate results. In fact, the results of our first blood sample from the two patients showed that smaller plasma DNA fragments of trisomy 18 were over the borderline Z-score and that fetal DNA fragments were greater than 10%. We requested temporary cessation of heparin injections on the morning of the second NIPT. In the second blood sample, the results were unaffected by the amount of short DNA fragments. Our results suggest that blood sampling for NIPT should be performed immediately before the next heparin injection, to avoid indeterminate NIPT results.

This study is limited by the small number of cases and the fact that it was conducted at a single center. Nevertheless, we were able to examine each patient's background in detail. The results indicate that we can provide satisfactory individualized genetic counseling to all patients undergoing NIPT in the future.

The Japan Society of Obstetrics and Gynecology began preparations for a new NIPT system in April 2022. Previously, women who requested NIPT without fetal abnormality were restricted to those older than 35 years, but this maternal age restriction will be removed in the future. It is expected that women with low-risk pregnancies will be more likely to receive NIPT in the new system. An analysis[11] of 146,958 pregnant women who received NIPT early in the second trimester included 40,287 women with low-risk pregnancies. The sensitivity, specificity, positive predictive value, and negative predictive value for aneuploidy (13, 18, 21 trisomies) were 98.97%, 99.95%, 81.36%, and 100%, and test accuracy was high for low-risk pregnancies. We expect that women with low-risk pregnancies will be more likely to request NIPT in the future, and the study outcomes are promising for such women. In addition, centers that cannot currently provide NIPT will be able to do so after submitting a new application. However, not all such facilities will have an adequate genetic counseling system. The Japan Society of Obstetrics and Gynecology recommends that NIPT be offered in cooperation with facilities that have advanced genetic counseling. Unfortunately, some pregnant women will need to go to other facilities to receive advanced genetic counseling after a positive NIPT result, which is inconvenient. Other pregnant women want access to advanced genetic counseling before NIPT or other perinatal genetic tests. We believe it is important for NIPT providers to acquire the knowledge necessary for genetic counseling and, above all, to have sufficient time to respond to the concerns and other needs of pregnant women at all facilities.

Conclusion

In our experience with NIPT over an 8-year period at our hospital, the positive rate, true-positive rate, and rate of indeterminate results for NIPT were generally similar to those reported in other Japanese studies. We believe that we can provide satisfactory individualized genetic counseling to all patients undergoing NIPT in the future.

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Conflict of Interest: None declared.

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