Implementation of non-invasive prenatal testing for aneuploidies in Slovenia

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Objective

Aneuploidies represent a major cause of perinatal death and childhood handicap. Consequently, the detection of chromosomal abnormalities constitutes the most frequent indication for invasive prenatal diagnosis. However, invasive procedures, such as amniocentesis or chorionic villus sampling (CVS), are associated with a 0. 5 – 1 % risk of fetal loss due to miscarriage. It is indicated only in pregnancies considered to be at high risk for aneuploidies. Screening by combination of fetal nuchal translucency, maternal serum free-β-human chorionic gonadotropin and pregnancy-associated plasma protein-A, can identify about 90% of fetuses with trisomy 21 and other major aneuploidies for a false-positive rate of 5% (1). Similar results were shown in Slovenia where detection rate is about 85% for a false-positive rate of less than 3% (2). Unfortunately, high rate of false positive screening results remains a major problem. In recent years, advances in molecular biology have enabled the development of highly accurate non-invasive prenatal tests (NIPT), based on cell-free fetal DNA (cffDNA) (3, 4). The discovery of cffDNA in maternal plasma in 1997 represents a key breakthrough to the further progress that has been made in the field of non-invasive prenatal testing (5). On average 10-20 % of cffDNA is present in the maternal plasma. The proportion varies strongly and it depends on different factors, such as gestational period and BMI of pregnant woman (6). In the last decade, several methods of NIPT for detecting chromosomal aneuploidies in early pregnancy have been developed. The massively parallel sequencing (MPS) method has been indicated as the most accurate, appropriate and therefore most commonly used. Clinical studies have shown that the method of MPS together with its variations is suitable as highly reliable screening for most common chromosomal aneuploidies in the first trimester of pregnancy (7, 8) and later in gestation. The experts agree that the NIPT method represents a highly accurate advanced screening test. Therefore in case of high risk result patients should still undergo one of the conventional invasive diagnostic procedures. First commercial NIPT tests were offered in USA at the end of 2011. In our institution we have performed the first NIPT test one year later. In this study we present implementation of NIPT testing and results in the clinic Diagnostični center Strah, Slovenia over the last 3 years.

Methods

123 participants were included in a retrospective observational study between 5. 2. 2013 and 20. 5. 2015. Samples were taken at the gestational age from 11th to 18th week of pregnancy. 120 women were pregnant with single child, 3 of them were carrying twins. Conventional prior screening tests were performed on all participants. 90 pregnant women had First trimester assessment risk, based on maternal age and fetal nuchal translucency. 31 women had combined screening test, based on maternal age, fetal nuchal translucency and biochemistry in the 1st trimester as well. 2 participants came for the NIPT with the First trimester risk assessment from other clinics. Indications for NIPT were advanced maternal age (35 years or older) or high risk result (cut-off 1/300), based on First trimester assessment of risk for trisomy 21, 18 and 13. In addition, some pregnant women with none of the high risk factors opted for NIPT on demand. Pretest counseling was provided to all the participants. Informed written consent was obtained before blood sampling. 10 mL of venous blood sample from each woman was used for NIPT molecular testing for T21, T18, T13 and sex chromosome aneuploidies. Samples were analyzed at BGI Diagnostic Laboratories. Pregnant women with high risk NIPT results were sent to genetic counseling and were advised to undergo the invasive diagnostic procedure. Pregnant women with low risk results underwent routine antenatal care, provided by their obstetricians. Telephone interviews were performed to women with high risk results in order to find out the outcome of pregnancy.

Results

NIPT has been performed on 123 pregnant women. 86 women (69. 9 %) were 35 years old or more (advanced maternal age). 21 women (17. 1 %) had high risk results for T21, T18 or T13, based on prior screening testing. 31 women(25. 2%) with no high risk factors decided to undergo NIPT (low risk population). High risk NIPT results were found in 6 cases including 3 cases of T21, 1 case of T18 and 2 cases of XXY. 5 of them were confirmed by subsequent fetal karyotyping, while case T18 was found as false positive. T13, XXX or X0 were not observed in any case. Furthermore, there were no false negative cases reported. Statistic characteristics (specificity, sensitivity …) were calculated based on 94 born children and 6 cases which were confirmed with diagnostic procedure. NIPT test shows 100 % sensitivity and 98. 95 % specificity. According to separate analysis, only for T21 NIPT was 100 % sensitive and 100 % specific. In 2015, the average turnaround time was 8. 3 days from the day when the sample was taken. Repeat blood sampling was required in 2 cases (redraw rate = 1. 6 %). 6 women out of 123, having high risk NIPT result underwent invasive diagnostic procedure (amniocentesis). In 5 of 6 cases, the high risk NIPT results were confirmed. One case was false positive. Without NIPT, 92 high risk pregnant women would undergo invasive diagnostic procedures (amniocentesis), which would result in 86 unnecessary amniocenteses.

Conclusion

We report our clinical study of implementation of non-invasive prenatal testing (NIPT) for most common fetal aneuploidies such as T21, T18, T13, XXY, XXX and X0, performed in a single centre. NIPT in our clinical study showed high sensitivity (100. 00 %) and specificity (98. 95%) for both high and low risk population of pregnant women. According only to T21 - Down syndrome, sensitivity and specificity were both 100. 00 %. Results are consistent with other validation and clinical studies, analyzing NIPT in other population groups (7, 8). In addition, our data shows a very low redraw rate (1. 6 %) and short turnaround time (8. 3 days). Without NIPT, amniocentesis or other invasive methods would be performed in all high risk pregnancies. According to our study population only 5. 4 % of high risk pregnant women (5 of 92) carried the fetus with chromosomal aneuploidy. 94. 6 % (87 of 92) of them would be exposed to the risk of fetal loss due to invasive diagnostic procedure. On the contrary in 83. 3 % (5 of 6) of total high risk NIPT the result was confirmed. One case (16. 7%) out of 6 high risk NIPT was found as false positive. Results of our study confirmed that NIPT represents a highly accurate non-invasive approach for screening Trisomy 21 and other most common aneuploidies. NIPT in routine clinical practice would significantly decrease the number of unnecessary diagnostic invasive procedures. Fetal loss, caused by invasive procedures, mostly amniocenteses, would significantly decrease as well.

Table : Epidemiological data for study population

Characteristic	Average	St. dev*	Median	Min	Max
Age	36.8	4.1	38	27	47
Weight	65.2	11.7	62	45	120
Height	168.1	5.2	168	155	180
Gravida	2	0.9	2	1	5
Twin pregnancy:			3 (2.4 %)		
Singleton pregnancy:			120 (97.6 %)		

** st.dev = standard deviation*

Table : Type of prior screening test

Prior test:	n (%)
1st trimester assessment of risk (other clinics)	2 (1.6 %)
1st trimester assessment of risk (age+ NT)	90 (73.2 %)
1st trimester assessment of risk (age+ NT+bioch)	31 (25.2 %)

Table : Indications for NIPT

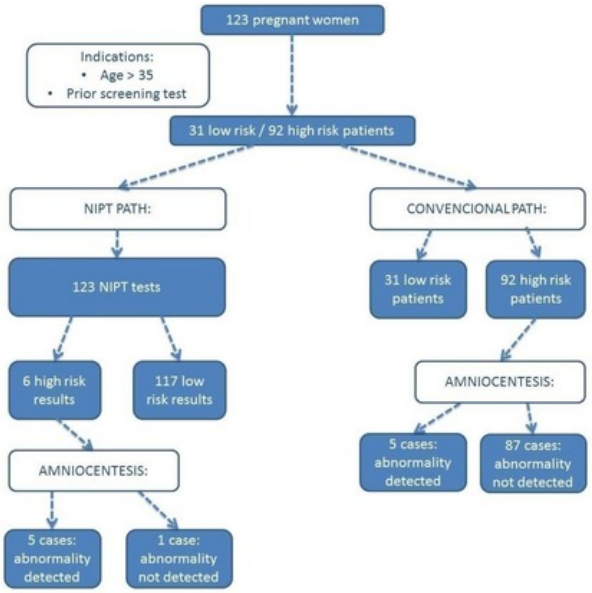
Indication	n (%)
High risk result at prior testing:	21 (17.1 %)
Age (35 and more):	86 (69.9 %)
High risk result at prior testing + age (35 and more):	15 (12.2 %)
No indication:	31 (25.2 %)

Table : High risk NIPT case characteristics

	Age	Prior T21 risk	Prior T18 risk	Prior T13 risk	NIPT result	Amniocentesis result
Patient 1	29	1 : 37	1 : 18265	1 : 18998	T21	T21
Patient 2	**43**	1 : **78**	1 : **821**	1 : **1656**	T21	T21
Patient 3	38	1 : 830	1 : 1624	1 : 828	T18	normal karyotype
Patient 4	**38**	1 : **3049**	1 : **7163**	1 : **20000**	**XXY**	**XXY**
Patient 5	41	1 : 874	1 : 977	1 : 4462	XXY	XXY (mosaic 90 %, normal 10 %)
Patient 6	**38**	1 : **784**	1 : **1860**	1 : **2660**	T21	T21

Table : NIPT test statistics

Average turnaround time in 2014	10.6 days			
Average turnaround time in 2015	**8.3 days**			
Redraw rate	2 (1.6 %)			
True positive	**5 (83.3 % of all high risk results)**			
False positive	1 (16.7 % of all high risk results)			
True negative	**94 (100 % of all low risk results and born yet)**			
False negative	0 (0 % of all low risk results and born yet)			
Specificity	**98.95 %**			
Sensitivity	100 %			
Positive predictive value	**83.3 %**			
Comparison between NIPT outcome in high risk (advanced age or prior testing) and low risk population				
Population	TP	FP	TN	FN
Low risk (no indication):	0	0	25	0
High risk (indication):	**5**	1	**69**	**0**



Picture 1: Comparison between NIPT screening and diagnostic path which include NIPT and hypothetic conventional path without NIPT.