Naslov prispevka:

SLO: NEINVAZIVNO PREDROJSTVENEGO TESTIRANJA ZA DOWNOV SINDROM IN OSTALE KROMOSOMSKE NAPAKE: KLINIČNA PRAKSA V SLOVENIJI

ANG: NON-INVASIVE PRENATAL TESTING FOR DOWN SYNDROME AND OTHER CHROMOSOMAL ABNORMALITIES: CLINICAL EXPERIENCE IN SLOVENIA

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Kratek opis članka:

SLO: 3 letna klinična praksa je potrdila, da z neinvazivnim predrojstvenim testiranjem (NIPT) z visoko občutljivostjo in specifičnostjo odkrijemo prisotnost Downovega sindroma in drugih kromosomskih napak pri plodu v zgodnji nosečnosti.

ANG: Clinical practise over last 3 years confirmed that non-invasive prenatal testing (NIPT) is a highly sensitive and specific method for detecting Down syndrome and other chromosomal abnormalities in early pregnancy.

Ključne besede:
SLO: ne-invazivno predrojstveno testiranje, kromosomske napake, Downov sindrom, nosečnost, fetalna DNK
ANG: non-invasive prenatal testing, chromosomal abnormalities, Down syndrome, pregnancy, fetal DNA

SLO:

Izhodišča: Amniocenteza trenutno predstavlja zlati standard predrojstvene diagnostike kromosomskih napak. Metoda je invazivna, neprijetna ter pri približno 0,5 – 1 % primerov vodi do izgube fetusa. Nasprotno s tem, neinvazivno pred-rojstveno testiranje (NIPT) analizira prosto fetalno DNK iz materine krvi in predstavlja visoko zanesljivo presejalno metodo za najpogostejše trisomije. V naši študiji predstavljamo rezultate NIPT testiranja v Diagnostičnem centru Strah v Sloveniji v zadnjih 3 letih.

Metode: V študijo je bilo vključenih 123 nosečnic med 11. in 18. tednom nosečnosti. Vsaki nosečnici je bil izveden predhodni presejalni test in NIPT testiranje.

Rezultati: 5 od skupno 6 primerov, katerim je NIPT pokazal visoko tveganje (vključujoč 3 primere T21 in dva primera XXY) je bilo potrjenih s kariotipizacijo med tem ko je bil 1 primer (T18) lažno pozitiven. Kromosomske napake T13, XXX ali X0 niso bile zaznane pri nobeni nosečnici. O lažno negativnih primerih ni bilo poročano. Glede na podatke, pridobljene v študiji, je NIPT testiranje v splošnem pokazalo 100 % občutljivost in 98,95 % specifičnost. Gledano le na T21 sta tako občutljivost kot senzitivnost 100 %. V letu 2015 je bilo povprečno trajanje analize 8,3 dni od dneva odvzema vzorca. V 2 primerih (1,6 %) je bil zaradi neuspešne analize potreben ponoven odvzem vzorca.

Zaključki: Naši rezultati potrjujejo, da NIPT predstavlja hitro, varno in visoko zanesljivo metodo za napreden presejalni test najpogostejših kromosomskih napak. NIPT bi v rutinski klinični praksi značilno znižal število nepotrebnih invazivnih postopkov in s tem tudi število spontanih splavov, ki jih invazivna diagnostika lahko povzroči.

ANG:

Background: Currently, amniocentesis still represents a gold standard for prenatal diagnosis of chromosomal abnormalities. The method is invasive, unpleasant and leads to a miscarriage in approximately 0.5 - 1 % of the cases. On the contrary, non-invasive prenatal testing (NIPT) analyses free fetal DNA from maternal blood and represent a highly accurate screening method for most common trisomies. In this study we present the results of NIPT testing in the clinic Diagnostični center Strah, Slovenia over the last 3 years.

Methods: In our study, 123 pregnant women from 11^{th} to 18^{th} week of pregnancy were included. On each patient prior screening tests were performed.

Results: 5 of total 6 high risk NIPT cases (including 3 cases of T21 and 2 cases of XXY) were confirmed by fetal karyotyping, 1 case (T18) was false positive. T13, XXX or X0 were not observed in any case. Furthermore, there were no false negative cases reported. In general, NIPT testing had 100 % sensitivity and 98.95 % specificity. Regarding only T21, specificity and sensitivity turned out to be 100 %. 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 %).

Conclusions: Our results confirmed that NIPT represents a fast, safe and highly accurate approach for advanced screening of most common aneuploidies. NIPT in routine clinical practice would significantly decrease the number of unnecessary invasive procedures and thus also the number of fetal loss, caused by invasive diagnostics.

INTRODUCTION

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 the 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 a highly reliable screening for most common chromosomal aneuploidies in the first trimester of pregnancy [4, 7] 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 the USA at the end of 2011. In our institution, we have performed the first NIPT test one year later. In this study, we present the 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. Epidemiological data are summarized in Table 1.

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 (Table 2).

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). Indications for NIPT are summarized in Table 3.

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 (Table 4). 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. Repeated 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). Characteristics (age and prior risks) are summarized in Table 5. 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, as it is shown on Picture 1.

DISCUSSION AND CONCLUSIONS

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 center. 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 show 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.

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Table 1: Epidemiological data for study population (123 pregnant women)

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*

Preglednica 1: Epidemiološki podatki za 123 nosečnic vključenih v študijo

Lastnost	Povprečje	St. dev*	Mediana	Minimum	Maksimum
Leta	36.8	4.1	38	27	47
Teža	65.2	11.7	62	45	120
Višina	168.1	5.2	168	155	180
Št. nosečnosti	2	0.9	2	1	5
Dvo-plodne nosečnosti:			3 (2,4 %)		
Eno-plodne nosečnosti:			120 (97,6 %)		

** st.dev = standardna deviacija*

Table 2: Type of prior screening test

Prior test:	$n_{pregnancies}$ (%)
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 %)

** NT = nuchal translucency*
**bioch = biochemistry markers*

Preglednica 2: Vrsta predhodnega presejalnega testa

Predhodni test:	$n_{nosečnic}$ (%)
Presejalni test v prvem trimesečju (druge klinike)	2 (1,6 %)
Presejalni test v prvem trimesečju (starost+NS)	90 (73,2 %)
Presejalni test v prvem trimesečju (starost+NS+biokem)	31 (25,2 %)

** NS = nuhalna svetlina*
**biokem = biokemijski markerji*

Table 3: Indications for NIPT

Indication	npregnancies (%)
High risk result at prior testing (> 1:300):	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 %)

Preglednica 3: Indikacije za NIPT

Indikacija	$n_{nosečnic}$ (%)
Visoko tveganje pri predhodnih presejalnih testih (> 1:300):	21 (17,1 %)
Starost (35 ali več):	86 (69,9 %)
Visoko tveganje pri predhodnih presejalnih testih (> 1:300) + starost (35 ali več):	15 (12,2 %)
Brez indikacije:	31 (25,2 %)

Table 4: 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

Preglednica 4: Statistika NIPT testiranja

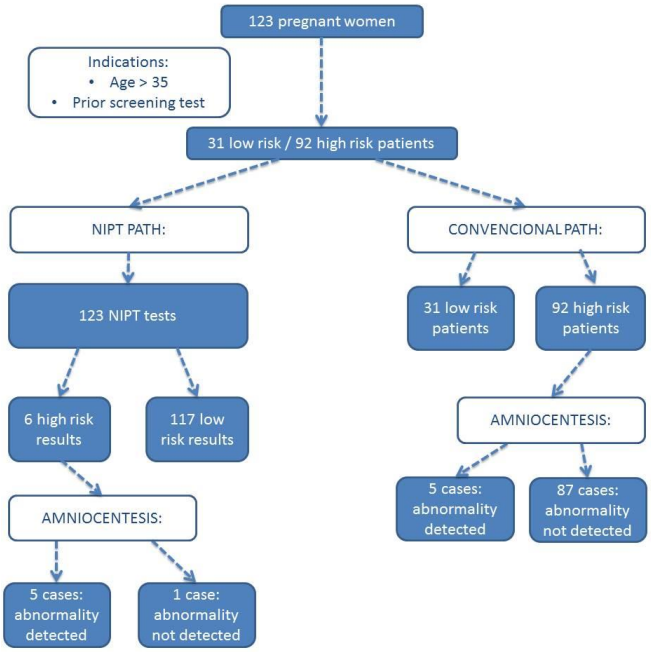
Povprečno trajanje analize v letu 2014	10,6 dni			
Povprečno trajanje analize v letu 2015	8,3 dni			
Zahtevana ponovna analiza	2 (1,6 %)			
Pravilno pozitivni (TP)	5 (83,3 % od vseh visoko rizičnih NIPT rezultatov)			
Lažno pozitivni (FP)	1 (16,7 % od vseh visoko rizičnih NIPT rezultatov)			
Pravilno negativni (TN)	94 (100 % od vseh nizko rizičnih NIPT rezultatov že rojenih otrok)			
Lažno negativni (FN)	0 (0 % od vseh nizko rizičnih NIPT rezultatov že rojenih otrok)			
Specifičnost	98,95 %			
Občutljivost	100 %			
Pozitivna napovedna vrednost	83,3 %			
Primerjava med NIPT rezultatom v visoko rizični (višja starost, predhodni presejalni testi) in nizko rizični populaciji				
Populacija	TP	FP	TN	FN
Nizko-rizična (brez indikacij):	0	0	25	0
Visoko-rizična (z indikacijo):	5	1	69	0

Table 5: 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 5: Značilnosti nosečnic z NIPT rezultatom: visoko tveganje

	Starost	$T21_{predhodno\ tveganje}$	$T18_{predhodno\ tveganje}$	$T13_{predhodno\ tveganje}$	NIPT rezultat	Rezultat amniocenteze
Nosečnica 1	29	1 : 37	1 : 18265	1 : 18998	T21	T21
Nosečnica 2	43	1 : 78	1 : 821	1 : 1656	T21	T21
Nosečnica 3	38	1 : 830	1 : 1624	1 : 828	T18	normalen kariotip
Nosečnica 4	38	1 : 3049	1 : 7163	1 : 20000	XXY	XXY
Nosečnica 5	41	1 : 874	1 : 977	1 : 4462	XXY	XXY (mozaik 90 %, narmalen 10 %)
Nosečnica 6	38	1 : 784	1 : 1860	1 : 2660	T21	T21



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