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Original Article

Study on the effect of non-invasive prenatal genetic testing on the application of invasive diagnostic testing

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Abstract: Objective: To explore the effect of non-invasive prenatal genetic testing (NIGPT) on the application of invasive chorionic villus sampling (CVS) or amniocentesis. Methods: A retrospective cohort study was conducted on two groups of pregnant women before (pre-NIPGT group) and after NIPGT (post-NIPGT group) applied clinically in 2011 and 2014, respectively. The two groups were compared to confirm whether the number of pregnant women who chose to receive invasive genetic testing presented significant differences. Results: The results of invasive genetic testing showed that about 29.4% of cytogenetic abnormalities could not be detected by NIPGT. Compared with the pre-NIPGT group, the number of pregnant women who received prenatal counseling and invasive genetic testing decreased insignificantly in the post-NIPGT group (1,778/2,598 vs. 2,520/3,722, P=0.39) but the number of pregnant women suggested to receive invasive genetic testing but gave up after receiving NIPGT evidently increased from 26.0% to 31.8% (P<0.01). Conclusion: Although NIPGT cannot reduce the invasive genetic testing rate significantly, the number of pregnant women who should receive invasive genetic testing, as they were not suitable for NIPGT, decreases leading to the risk of missed diagnosis.

Keywords: Non-invasive prenatal genetic testing, chromosomal aneuploidy mutation, first-trimester screening, chorionic villus sampling, amniocentesis

Introduction

Non-invasive prenatal genetic testing (NIPGT) can effectively screen whether the pregnant women's fetal are high-risk carriers of chromosomal aneuploidy mutation (such as Down syndrome) by detecting their peripheral blood [1]. In the past few decades, prenatal screening for Down syndrome has developed slowly. Since October 2011, with the discovery of cell-free DNA in maternal peripheral blood and rapid development of gene sequencing technology, NIPGT has been widely applied clinically to detect fetal chromosomal aneuploidy mutations [2]. NIPGT is intended to detect fetal cell-free DNA in maternal peripheral blood, which is a prenatal screening with great potential. A growing number of studies have confirmed that non-invasive fetal aneuploidy chromosomal testing achieved an accuracy of above 99% for trisomy 21 syndrome and trisomy 18 syndrome, as well as up to 91% for trisomy 13 syndrome [3-5]. It is considered to be the best technical method for detecting trisomy 21 syndrome and trisomy 18 syndrome [6]. A study on the factors influencing clinical application of NIPGT showed that pregnant women chose NIPGT because of less trauma, low risk, and high safety to fetal [7]. However, a recent study found that although the accuracy of NIPGT in disease diagnosis is still questionable, the number of pregnant women with positive genetic abnormalities who should receive further invasive genetic testing has decreased significantly with the popularization of NIPGT [8].

Therefore, this retrospective cohort study was conducted on the pregnant women who received prenatal counseling and testing a year before and a year after clinical NIPGT application, in view of positive perceptions and high expectations of the patients for NIPGT and excessive fear of invasive genetic testing [6, 7, 9, 10]. According to the application status of NIPGT, the difference between the number of pregnant women who chose to receive NIPGT

Table 1. Indications of pregnant women who received prenatal counseling and examinations (n, %)

Indications of prenatal counseling and examinations*	3,722 (Year 2011)	2,598 (Year 2014)	P
Advanced age	3,204 (86.1)	1,969 (75.8)	0.01
Not commend NIPGT			
Monogenic mutation	104 (2.8)	42 (1.6)	0.03
Balanced translocation carrier	21 (0.6)	13 (0.5)	0.47
Multiple pregnancies	301 (8.1)	231 (8.9)	0.39
Twin-twin transfusion syndrome	22 (0.6)	10 (0.4)	0.46
Total number of not recommend NIPGT	448 (12.0)	296 (11.4)	0.13
Gave up invasive examination after NIPGT	968 (26.0)	825 (31.8)	<0.01
Noninvasive screening			
Early and mid-term pregnancy examination	692 (18.6)	901 (34.7)	<0.01
NIPGT	0	602 (23.2)	
Invasive genetic testing			
Chorionic villus sampling	2,208 (59.3)	1,594 (61.3)	0.57
Amniocentesis	312 (8.4)	184 (7.1)	0.45
All means of invasive genetic testing	2,520 (67.7)	1,778 (68.4)	0.39

Note: NIPGT, non-invasive prenatal testing; *some pregnant women might have more than two indications.

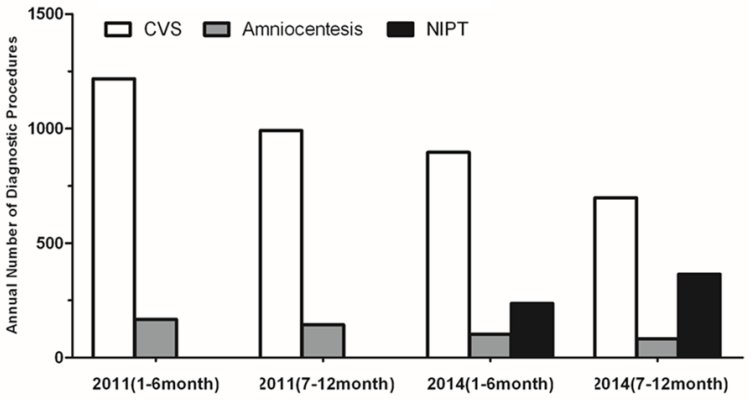

Figure 1. Comparison of the number of pregnant women who received prenatal examinations within different periods. CVS, chorionic villus sampling; NIPGT, non-invasive prenatal testing.

Table 2. Summary of results of invasive genetic testing within the two periods (n, %)

Total cases of invasive genetic testing	n=4,298
No cytological abnormalities	4,053 (94.3)
Cytological abnormalities	245 (5.7)
Chromosomal aneuploidy mutation	142 (58.0)
Sex chromosome abnormalities	31 (12.6)
Other (cannot detect by NIPGT)	72 (29.4)

Note: NIPGT, non-invasive prenatal testing.

and invasive diagnosis in two experimental groups was compared. Also, the means of choosing a safe and reliable prenatal diagnosis was explored to provide reference for further clinical research and application.

Materials and methods

Study objects

This study was approved by the Hospital Ethics Committee and all of the subjects had signed the informed consent. The pregnant women who received prenatal counseling and testing in Maternal and Child Health Hospital of Zibo City between January 2011 and December 2011 (pre-NIPGT group) and between January 2014 and December 2014 (post-NIPGT group) were enrolled in this study. They had an average age of 26.02 and 26.74 and gestation of 12-20 weeks ± 5 days and 11-19 weeks ± 6 days.

Inclusion criterion: subjects aged ≥ 18.

Exclusion criteria: subjects aged below 18; subjects with genetic abnormalities or family history; subjects with medical history and incomplete data records.

Study materials

The collected data included the number of pregnant women who received prenatal counseling and genetic diagnosis; the number of pregnant women who were suitable to receive invasive prenatal genetic testing but gave up

Table 3. Comparison of results of invasive genetic testing within the two periods

	Year 2011	Year 2014	P
Case	2,520	1,778	
No cytological abnormalities	2,387 (94.7)	1,666 (93.7)	0.73
Cytological abnormalities	133 (5.3)	112 (6.3)	0.10
Chromosomal aneuploidy mutation	75 (56.4)	67 (59.8)	0.27
Sex chromosome abnormalities	17 (12.8)	14 (12.5)	0.82
Other	41 (30.8)	31 (27.7)	0.71

and the number of pregnant women who were not suitable to receive NIPGT.

Indications for the pregnant women were not suitable to receive NIPGT: monogenic mutation, balanced translocation carrier, multiple pregnancies, and twin-twin transfusion syndrome.

All of the patients received invasive genetic testing (chorionic villus sampling (CVS) and amniocentesis). Testing was performed in Maternal and Child Health Hospital of Zibo City.

Statistical analysis

Statistical analysis was conducted using SPSS21.0. Categorical variables were expressed as percentage and the difference between groups was compared by Chi-square test. $P<0.05$ represents a statistically significant difference.

Results

A total of 3,722 pregnant women received prenatal counseling and genetic diagnosis between January 2011 and December 2011. The number decreased to 2,598 between January 2014 and December 2014, a decrease of 30.2% (**Table 1**) and the percentage of the women with advanced maternal age who received prenatal counseling and genetic diagnosis also decreased significantly (86.1% vs. 75.8%, P=0.01). Among the patients who were not suitable to receive NIPGT, the percentage of patients with monogenic mutation decreased from 2.8% to 1.6% (P=0.03) while the percentage of patients with balanced translocation, multiple pregnancies, and twin-twin transfusion syndrome presented a statistically significant difference (all P>0.05). In addition, the percentage of pregnant women suggested to receive invasive genetic testing but gave up after received NIPGT increased from 26.0% to 31.8% (P<0.01). The study data showed that the percentage of pregnant women who received invasive genetic testing presented no significant change within the two periods. However, the percentage of pregnant women who received invasive genetic testing decreased year by year as the percentage of the pregnant women who received NIPGT increased. See **Figure 1**.

A total of 4,298 pregnant women underwent invasive genetic testing in 2011 and 2014, in which 29.4% of cytogenetic abnormalities could not be detected by NIPGT (**Table 2**). The results of invasive genetic testing within the two periods presented no statistically significant difference in the detection rates (**Table 3**). The genetic abnormalities that could not be detected by NIPGT are listed (**Table 4**).

Discussion

NIPGT is generally used to detect chromosomal aneuploidy mutations but its coverage is incomplete, therefore, some cytogenetic abnormalities can only be diagnosed by invasive means, such as CVS and amniocentesis [11]. For invasive genetic testing, complete fetal cells are obtained so that the fetal chromosome karyotype can be analyzed. Meanwhile, DNA is provided for array-based comparative genomic hybridization (CGH), so that triploid syndrome and non-balanced translocation carriers can be identified accurately through identification of karyotype, as well as obvious sequence copy exception (microdeletion and microduplication) and related diseases can be diagnosed accurately [12-14]. Our study showed that nearly 29.4% of cytological abnormalities could not be detected simply by extracting a bit of peripheral blood, similar to that of a previous study [15]. A recent study reported 109 NIPGT-positive pregnant women who underwent invasive genetic testing; the true positive rates of trisomy 21 syndrome, trisomy 18 syndrome, trisomy 13 syndrome and sex chromosome abnormality were 93.0%, 64.0%, 44.0% and 38.0%, respectively [16]. These results indicate that the false positive rates of trisomy 18 syndrome, trisomy 13 syndrome,

Table 4. Summary of cytological abnormalities that could not be detected by NIPGT within the two periods (detected by invasive genetic diagnosis)

	Chromosomal abnormalities	Total number (2011 and 2014)
Chromosome number abnormalities	7-trisomy syndrome	6
	8-trisomy syndrome	6
	9-trisomy syndrome	2
	10-trisomy syndrome	1
	12-trisomy syndrome	2
	15-trisomy syndrome	1
	16-trisomy syndrome	1
	20-trisomy syndrome	1
	22-trisomy syndrome	1
Chromosomal structural abnormalities	Balanced chromosomal translocation	19
	Non-balanced chromosomal translocation	7
	Chromosome deletion	4
	Chromosome duplication	7
	Marker chromosome	5
	Isochromosome	3
	Chromosome inversion	5
	Circular chromosome	1
Total		72

Note: NIPGT, non-invasive prenatal testing.

and sex chromosome abnormality are as high as 36.0%, 56.0% and 62.0%, respectively, highlighting the limitations of NIPGT.

A recent study analyzed the problem that NIPGT should be defined as a screening or diagnostic means from the perspective of cost efficiency and then compared the differences between the two. They found that if NIPGT was regarded as the independent diagnostic method of trisomy 21 syndrome instead of invasive genetic diagnosis, it would raise the birth rate of children with Down syndrome as well as the percentage of pregnant women with pregnancy termination. In addition, the odinopoeia risk of non-high risk fetus was over 100 times higher than the risk of abortion caused by invasive operations [17]. In summary, no matter how high the detection rate of NIPGT is, it is still a screening method with the risk of false positive and false negative detection rates.

The results of our study indicate that the percentage of pregnant women who choose prenatal counseling or genetic diagnosis because of monogenetic mutation present the most remarkable decline after application of NIPGT, while monogenetic mutations could not be detected by NIPGT. It means that high-risk pregnant women with fetal chromosomal aneuploidy mutation and with monogenic mutation may be recommended to receive NIPGT by the initial doctor, who may ignore the benefit-risk balance between non-invasive screening and invasive diagnosis. For non-high-risk pregnant women who choose NIPGT, the positive and negative predictive values are also worthy of concern. The positive predictive value will fluctuate as the prevalence rate changes. For instance, the positive predictive value of NIPGT for a pregnant woman with 1% of Down syndrome risk is 67% and it will decrease to below 20% if the risk falls to 1/1,000 [18].

Currently, more and more pregnant women choose NIPGT while the applications of first-trimester screening and invasive genetic testing (such as CVS and amniocentesis) have been limited greatly. This may possibly make the application of invasive diagnostic means decline gradually until disappearing [19]. Most patients will selectively ignore the safety of invasive genetic diagnosis and the misdiagnosis rate of NIPGT. Therefore, it is the perceived risk rather than a credible theoretical basis

that plays a decisive role in patient choice of invasive diagnosis or NIPGT [20].

The large outpatient volume and reliable prenatal genetic testing technology of the Department of Gynecology & Obstetrics in our hospital contributed to the largest advantage of this study, but some limitations are applicable. This was a retrospective study. Only the patients in our hospital were included and the study period was also limited, thus causing inevitable selection and information bias in the samples. Therefore, we could not conclude that patients in other hospitals hold the same attitude toward and choice of prenatal genetic diagnosis.

In conclusion, our data show that after NIPGT has been applied clinically, the percentage of pregnant women suggested to receive invasive genetic testing but gave up after received NIPGT increases. Nevertheless, NIPGT has its limitations. Before more accurate non-invasive genetic testing is proposed for clinical application, invasive genetic diagnosis should be extensively applied in high-risk pregnant women in order to reduce missed diagnoses and misdiagnosis rates.

Disclosure of conflict of interest

None.

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