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Original Article

Are the selected criteria of NIPT reasonable? New point of view from the analysis of the Down syndrome characteristics

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Abstract: Aims: To discuss the selected criteria of NIPT according to the data of Down syndrome (DS) characteristics. Methods: In this multicenter study, we collected a total of 332 cases of DS in three centers (206 cases were prenatal diagnosed and 126 miss diagnosed). We collected the mothers' blood and detected the levels of AFP and fβhCG by TRFIA, and calculated the risk value of trisomy 21. Sensitivity, specificity, PPV and NPV were analysed by receiver operation characteristic curve (ROC curve). Results: The estimated detection rate was 62.0% (206/332) for DS in the second trimester. We evaluated the significance of trisomy 21 risk value to judge whether the fetus suffer from DS or not by ROC curve, and the AUC was 0.9581. There were two important cut-off trisomy 21 risk values to be noted: 1/365 and 1/1050. 1/1050 was the optimal cut-off value with the sensitivity, specificity, PPV and NPV were 88.3%, 88.0%, 88.8% and 87.5%, respectively. The PPV and specificity were both 100% when trisomy 21 risk value was 1/365. 67.1% DS cases whose trisomy 21 risk value were higher than 1/365, and 88.2% were higher than 1/1050. However, 88.0% cases in normal control group were lower than 1/1050. No case from normal control group would be higher than 1/365. Conclusion: Analyze the clinical features of Down syndrome is necessary to use NIPT more reasonably.

Keywords: Down syndrome, serum, prenatal screening, non-invasive prenatal testing, prenatal diagnosis, cell-free DNA

Introduction

Down syndrome (DS) is one of the most common types of gross chromosomal abnormalities, with an incidence of 1 in 700 [1]. It is well known that prenatal screening and diagnosis are the only way to avoid the birth of DS baby. In the past three decades, prenatal screening for DS has been widely used in pregnant women [2, 3]. The most common prenatal screening method for DS is a combination of serum levels of alpha-fetoprotein (AFP), unconjugated estriol (uE3), the free beta subunit of human chorionic gonadotropin (fβhCG) and maternal age in the second trimester [4, 5]. The rate of detection (DR) of DS is 75% with a 5% false-positive rate (FPR) by this screening program [6]. However, the DR is much lower in some developing countries. In China, the DR is only 50%~67% with a 5% FPR according to a multi-center study [7-9]. Recently, prenatal screening in first trimester has also been widely used. Using this screening program, 75%~85% DS fetuses could be detected with a FPR of 5% [10-12]. The estimated DR was reported as 87.0% for DS and 91.8% for trisomies 18 and 13, at a FPR of 2.2% [13]. However, some DS cases still missed diagnosis due to the technical limitations of the prenatal screening. So the DR of the prenatal screening program still need to improve.

Recently, non-invasive prenatal testing (NIPT) for common fetal aneuploidies was proved to be a better prenatal screening program, which detected cell-free fetal DNA (cfDNA) obtained from maternal plasma by massively parallel

Table 1. The baseline characteristics of three groups in this study

	T21 prenatal diagnosed group	T21 miss diagnosed group	Normal control group	*P*-value
N	206	126	309	
Ethnicity (Chinese)	206	126	309	
Singleton pregnancy	206	126	309	
Maternal Age (years)	29.90 ± 5.42	27.64 ± 4.18	28.00 ± 3.71	<0.001
Maternal Weight (kg)	56.94 ± 8.32	57.23 ± 7.87	58.88 ± 10.47	0.056
Gestational age (weeks)	16.70 ± 1.00	16.86 ± 1.16	16.88 ± 0.95	0.112

Data were analyzed by ANOVA test.

sequencing (MPS). Nowadays, NIPT was widely used to prenatal screen the T21, T18, T13 and presented good accuracy [14]. The American College of Obstetricians and Gynecologists (ACOG) [15], International Society for Prenatal Diagnosis (ISPD) [16] have issued the committee opinions or guidelines about the clinical application of NIPT, and they both recommend the patients of high risk group to accept NIPT. In 2015, Chinese scholars firstly suggested the mothers whose T21 screening results were intermediate risk (1/270~1/1000) to accept NIPT to reduce the missed diagnosis. However, is it reasonable? Should we adjust more reasonable range of NIPT?

In the present study, we collected DS case who were prenatal diagnosed or miss diagnosed from three prenatal diagnosis centers and analyzed their characters. By statistical analysis, we hope to improve the accuracy of prenatal screening programs and reduce the missed diagnosis of DS.

Materials and methods

Patients and design

This multicenter study was conducted in the Changzhou Women and Children Health Hospital of Nanjing Medical University, Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University and Suzhou Municipal Hospital (China). The cases of this study were comprised of pregnant women who accepted prenatal screening from October 2002 to June 2015. A total of 332 DS cases were collected, including 206 prenatal diagnosed be prenatal diagnosed and 126 unsuccessfully diagnosed. We selected their mothers as the objects in this research. Meanwhile, 309 mothers who had normal babies were selected as the normal control group. The common parameters are shown in **Table 1**.

The study design and protocol were reviewed and approved by the ethics committee of Changzhou Women and Children Health Hospital affiliated to Nanjing Medical University.

Methods

Samples collect: All of the subjects received the prenatal screening in second trimester after genetic counseling and informed consent. According to the operating program of prenatal screening, we collected the blood of every case in second trimester (15 w~20 w). Gestational age was calculated by each pregnant woman's last menstrual period or ultrasonography. 3 ml blood of all the cases were collected by simple needle aspiration. After being placed 0.5 h at room temperature, the samples were centrifuged at 3000 rpm for 5 minutes to remove cells. The serum was stored at 4°C until assays within 7 days and long-stem stored at -80°C.

Prenatal screening in second trimester: As Miao [17] described, the levels of AFP and fβhCG were quantified by time-resolved fluoroimmunoassay (TRFIA) using Wallac 1235 AutoDELFIA (DELFIA1235: Perkin Elmer, Waltham, MA). The values were also presented as multiples of the median (MoM) and determined the risk of DS with Wallace LifeCycleTM Elipse analysis software (Perkin Elmer). The current cut off value of trisomy 21 risk were 1/270 and 1/1000. Higher than 1/270 was account as high risk, and 1/270~1/1000 was account as intermediate risk. Advanced age: maternal age ≥ 35.

Statistical analysis

The stratified analysis, the interaction test, covariate screening, curve fitting, and the receiver operating characteristic curve (ROC curve) were performed using EmpowerStats x64 software [18]. P<0.05 was chosen to be statisti-

Table 2. Compared the value of fβhCG and AFP between three groups

	T21 prenatal diagnosed group	T21 miss diagnosed group	Normal control group	*P*-value
fβhCG (ng/ml)	53.60 (37.80-78.30)	21.85 (15.50-30.65)	13.90 (9.36-21.88)	<0.001
HCG MoM	3.74 (2.54-5.21)	1.66 (1.19-2.10)	0.91 (0.63-1.42)	<0.001
AFP (U/ml)	27.52 ± 12.99	32.58 ± 12.42	42.97 ± 15.15	<0.001
AFP MoM	0.75 ± 0.39	0.84 ± 0.32	1.10 ± 0.36	<0.001

Note: The normal distribution data were expressed as mean ± SD. The abnormal distribution data were expressed as median (Q1-Q3). Data were analyzed by ANOVA test to compare normally distributed data and with Kruskal Wallis Rank Test to compare non-normal distributions.

Table 3. Association of fβhCG and AFP levels with DS

	Total	Odds ratios*	95% CI	*P* value
fβhCG (ng/ml)	22.39 (12.80-41.33)	1.1	1.1-1.1	<0.001
HCG MoM	1.52 (0.86- 2.76)	6.8	4.9-9.4	<0.001
AFP (U/ml)	36.14 ± 15.61	0.9	0.9-0.9	<0.001
AFP MoM	0.94 ± 0.40	0.1	0.0-0.1	<0.001

Note: *Adjust for: AGE; WEIGHT, Gestational age. Odds ratios and 95% confidence intervals are presented to show the risk of Down's syndrome. Data were analyzed by multivariate logistic analysis, model X one by one with concomitant variable.

cally significant. Results of parameters were expressed as mean ± SD for continuous variables with normal distribution, median (M), 2.5th percentile (P2.5) and 97.5th percentile (P97.5) for the data with abnormal distribution. Analysis of Variance and non-parametric test were employed to compare differences for continuous variables between the groups.

Results

General results of prenatal screening for DS

In the past 13 years, we collected 332 DS cases whose mothers accepted prenatal screening program. Among 332 cases, 206 were prenatal diagnosed by amniocentesis or percutaneous umbilical blood sampling after serum screening. However, a total of 126 DS babies have still been missed diagnosis in three prenatal diagnosis centers. The estimated DR was 62.0% (206/332) for trisomies 21 in the second trimester.

Relationship between fβhCG, AFP and DS failed to be diagnosed

Compared with normal control group, the level of fβhCG and HCG MoM were significant increased in T21 group (both in T21 prenatal diagnosed group and T21 miss diagnosed group), while AFP and AFP MoM decreased significantly, as shown in **Table 2**. The difference was still existed in the comparison between the T21 prenatal diagnosed group and T21 miss diagnosed group. By logistic regression analysis after adjusted for maternal age and weight, the odds ratios (OR) and 95% confidence intervals were shown in **Table 3**. HCG-MoM was one of the risk factor of DS (OR=6.8), while the OR of AFP-MoM was 0.10. Based on the changes of HCG-MoM and AFP-MoM, we calculated their primary outcome for trisomy 21 screening by the area under the ROC curve (AUC). As shown in **Figure 1**, the AUC for trisomy 21 was 0.9581 and the sensitivity, specificity, positive predictive value and negative predictive value were 88.3%, 88.0%, 75.4% and 83.6%, respectively.

Relationship between Risk and DS failed to be diagnosed

After analyzed the distribution of the groups according to T21-risk by ROC test, two important cut-off value needed to be noted: 1/365 and 1/1050. 1/1050 was the optimal cut-off value with the sensitivity, specificity, PPV and NPV were 88.3%, 88.0%, 88.8% and 87.5%, respectively. While the PPV and specificity of 1/365 were both 100%. So we compared the distribution of the groups by 1/365 and 1/1050, as shown as **Table 4**.

It was worthwhile noted that the risk of 67.1% trisomy 21 cases were higher than 1/365, 88.2% higher than 1/1050. However, 88.0% cases in normal control group were lower than 1/1050. No case from normal control group was higher than the value of risk in 1/365. Meanwhile, 69.1% T21 miss diagnosed cases were higher than 1/1000. In China, the mothers with the T21-risk between 1/270 and

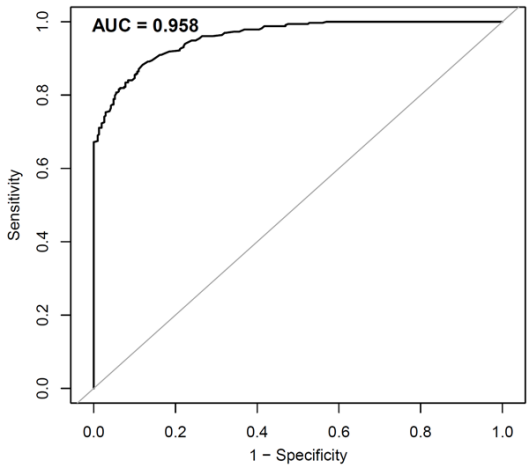

Figure 1. The significance of trisomy 21 risk value to judge whether the fetus suffer from DS or not was analyzed by ROC test.

1/1000 were suggested to accept NIPT. If we chose NIPT follow this standard, 88.2% T21 cases could be diagnosed, while 11.7% might be missed. No cases was between 1/1000 and 1/1050 in present study, although we found that 1/1050 was the optimal cut-off value.

Discussion

It is well known that prenatal screening contribute to avoid the birth of DS babies. However, it needs to be improved greatly. Although we have tried our best in screening, the estimated DR was 62.0% (206/332) for trisomies 21 in the second trimester, a total of 126 DS babies were unfortunately missed diagnosis in three prenatal diagnosis centers. We aimed to improve the efficiency of prenatal screening programs by investigating the characters of DS.

At first, we found that the levels of fβhCG and HCG MoM were dramatically increased, while AFP and AFP MoM decreased in DS group. Meanwhile, the difference was more significant in the T21 prenatal diagnosed group. In the common prenatal screening program, we calculated the T21-risk combined with maternal age, gestational week, the levels of fβhCG and AFP. However, maybe we could make use of them better to improve the accuracy of prenatal screening program. As shown in our result, the area under the ROC for primary outcome of trisomy 21 screening was 0.9581 for HCG-MoM and AFP-MoM testing. Maybe we can use these indexes better to help reduce the DS cases who be missed diagnosis.

Second, it was worthwhile noting that the characteristic of the distribution according to T21-risk. It is well known that the value of risk is the most important index in prenatal screening program, and it also used to determine whether these pregnant women accept NIPT or not. ACOG [15] and ISPD [16] both recommend the high risk population to accept NIPT. Chinese scholars firstly suggested the mothers whose DS screening results were intermediate risk (1/270~1/1000) to accept NIPT. According to the results of present study, we recommended paying attention to the two cut-off: 1/365 and 1/1050. In the past, 1/270 was used as the basis of T21 high risk judgment. While we found that the PPV and the accuracy of 1/365 was both 100% and 100%. If we choose the follow-up treatment by the traditional criteria (1/270), 37 DS cases with the risk between 1/271 and 1/365 might be failed to be diagnosed. Meanwhile, the risk of 67.1% DS cases were higher than 1/365, while no normal pregnant women could be higher than 1/365. It meant that if the T21-risk of a pregnant women was higher than 1/365, the risk of her fetal of DS was very high. So the women should be recommended to accept the cytology of prenatal diagnosis, such as amniocentesis or pencutaneous umbilical blood sampling. Although 1/1050 was the optimal cut-off value, the T21-risk of 88.0% normal pregnant women were lower than 1/1050. However there were still 11.7% T21 cases in the interval. The present point suggest the women whose T21-risk was higher than 1/1000 should accept NIPT. In present study, no cases were in the interval between 1/1000 and 1/1050. Whether the standard of 1/1050 is more effective? It needs more clinical data validation in the further. For the pregnant women whose risk value were between 1/365 and 1/1050, NIPT is a good choice.

In conclusion, we collected the DS cases from three prenatal diagnosis centers and analyzed their characters to help us reduce the missed diagnosis. It is also necessary to adjust more reasonable range of NIPT with further clinical researches.

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Table 4. Compared the distribution of the groups according to T21-risk, n (%)

	All DS, n (%)	T21 miss diagnosed group, n (%)	T21 prenatal diagnosed group, n (%)	Normal control group, n (%)
>1/270	186 (56.0)	1 (0.8)	185 (89.8)	0
1/271~1/365	37 (11.1)	20 (15.9)	17 (8.3)	0
1/366~1/1000	70 (21.1)	66 (52.4)	4 (1.9)	37 (12.0)
1/1001~1/1050	0	0	0	0
<1/1051	39 (11.7)	39 (31.0)	0	272 (88.0)
Total	332 (100)	126 (100)	206 (100)	309 (100)

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Disclosure of conflict of interest

None.

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References

[1] Roizen NJ, Patterson D. Down's syndrome. Lancet 2003; 361: 1281-1289.

[2] Wald NJ. Prenatal screening for open neural tube defects and Down syndrome: three decades of progress. Prenat Diagn 2010; 30: 619-621.

[3] Maxwell S, Bower C and O'Leary P. Impact of prenatal screening and diagnostic testing on trends in Down syndrome births and terminations in Western Australia 1980 to 2013. Prenat Diagn 2015; 35: 1324-1330.

[4] Cuckle HS, Wald NJ and Thompson SG. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. Br J Obstet Gynaecol 1987; 94: 387-402.

[5] Wald NJ, Cuckle HS, Densem JW, Nanchahal K, Royston P, Chard T, Haddow JE, Knight GJ, Palomaki GE and Canick JA. Maternal serum screening for Down's syndrome in early pregnancy. BMJ 1988; 297: 883-887.

[6] Spencer K. Second trimester prenatal screening for Down's syndrome using alpha-fetoprotein and free beta hCG: a seven year review. Br J Obstet Gynaecol 1999; 106: 1287-1293.

[7] Wang YY, Luo J, Zhu MW, Liu LN and Ma X. Second-trimester double or triple screening for Down syndrome: a comparison of Chinese and Caucasian populations. Int J Gynaecol Obstet 2006; 94: 67-72.

[8] Liu QL, Hu YL, Xu ZF, Wang LJ, Sun Q, Lin N, Xu XY, Liu Y, Zhang JW, Tong JS, Wang XH and He J. A study on population-based prenatal screening and diagnosis of Down's syndrome in Jiangsu province. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2010; 27: 340-342.

[9] Hu YL; Birth Defect Intervention Group of Jiangsu Province. Serum screening of fetal chromosome abnormality during second pregnancy trimester: results of 26,803 pregnant women in Jiangsu Province. Zhonghua Yi Xue Za Zhi 2007; 87: 2476-2480.

[10] Cuckle H, Benn P and Wright D. Down syndrome screening in the first and/or second trimester: model predicted performance using meta-analysis parameters. Semin Perinatol 2005; 29: 252-257.

[11] Schielen PC, van Leeuwen-Spruijt M, Belmouden I, Elvers LH, Jonker M and Loeber JG. Multi-centre first-trimester screening for Down syndrome in the Netherlands in routine clinical practice. Prenat Diagn 2006; 26: 711-718.

[12] Wright D, Spencer K and Nix B. First trimester screening for Down syndrome using free beta hCG, total hCG and PAPP-A: an exploratory study. Prenat Diagn 2007; 27: 1118-1122.

[13] Wright D, Syngelaki A, Bradbury I, Akolekar R and Nicolaides KH. First-trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemical testing. Fetal Diagn Ther 2014; 35: 118-126.

[14] Gil MM, Quezada MS, Revello R, Akolekar R and Nicolaides KH. Analysis of cell-free DNA in

maternal blood in screening for fetal aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol 2015; 45: 249-266.

[15] Committee Opinion No. 640: cell-free DNA screening for fetal aneuploidy. Obstet Gynecol 2015; 126: e31-7.

[16] Benn P, Borrell A, Chiu RW, Cuckle H, Dugoff L, Faas B, Gross S, Huang T, Johnson J, Maymon R, Norton M, Odibo A, Schielen P, Spencer K, Wright D and Yaron Y. Position statement from the chromosome abnormality screening committee on behalf of the board of the international society for prenatal diagnosis. Prenat Diagn 2015; 35: 725-734.

[17] Miao ZY, Liu X, Shi TK, Xu Y, Song QH and Tang SH. First trimester, second trimester, and integrated screening for Down's syndrome in China. J Med Screen 2012; 19: 68-71.

[18] Wu J, Geng J, Liu L, Teng W, Liu L and Chen L. The relationship between estimated glomerular filtration rate and diabetic retinopathy. J Ophthalmol 2015; 2015: 326209.