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

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

Data were analyzed by ANOVA test.
Analysis the characteristics of DS cases

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

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

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

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

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.

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
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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.

Acknowledgements

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

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

analysis. This study was supported by Key research and development plan project of Jiangsu Province (BE2017650), Changzhou science and technology support project (Social Development CE20175021), Project funding for the training of high level health professionals in Changzhou (2016CZLJ013), Suzhou Science and Technology Support Program (SS201429), Suzhou Key Medical Center (SZZX201505), Jiangsu Maternal and Children health care research project (F201603), Jiangsu Provincial Medical Innovation Team (CXTDB2017013).

Disclosure of conflict of interest

None.

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