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ORIGINAL ARTICLE

Analysis of Prenatal Serological Screening of 116 Pregnant Women with Trisomy 21 Syndrome Fetus

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SUMMARY

Background: **Prenatal serological screening is commonly used to screen for trisomy 21 syndrome (T21); however, it carries a risk of missed detection. In this study, we explored how to reduce missed diagnosis of T21 in serological screening.**
Methods: **A total of 116 pregnant women with T21 fetuses confirmed by prenatal diagnosis were evaluated. Serological screening and non-invasive prenatal test (NIPT) findings were analyzed.**
Results: **Twenty-nine T21 fetuses were missed in serological screening; the missed diagnosis rate was 25%, 67.65% of the missed cases were of moderate risk, and 79.31% of the missed pregnant women were under 35 years of age. Nuchal translucency (NT) and the free beta subunit of human chorionic gonadotropin (free-HCGβ) were higher in the detected T21 cases than in the missed T21 cases, while alpha-fetoprotein (AFP) levels were decreased. Forty-eight pregnant women who underwent NIPT in the second trimester were at high-risk for T21.**
Conclusions: **Prenatal screening should not be ignored in young pregnant women. For serological screening, moderate risk and abnormal single serum markers should also receive greater attention. NIPT can be extended to first-tier screening.**
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KEYWORDS

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INTRODUCTION

Among all birth defects, chromosomal diseases are one of the more serious. Trisomy 21 (T21) has the highest incidence rate and children with T21 have intellectual disabilities accompanied by various systemic abnormalities [1]. There are no effective, targeted, and economic treatments to completely cure such diseases; thus, early intervention through prenatal screening and prenatal diagnosis is the main prevention and control method to give the couple all the possible information and allow them to make a decision [2].
Since the 1990s, prenatal serological screening has been gradually applied. This method detects the free beta

subunit of human chorionic gonadotropin (free-HCGβ) and pregnancy-associated plasma protein A (PAPP-A) in the first trimester or alpha-fetoprotein (AFP), unconjugated estriol (uE3), and free-HCGβ in the second trimester, combined with factors such as nuchal translucency (NT), maternal age, maternal weight, and gestational age, to obtain the risk of fetal T21, trisomy 18 (T18), and open neural tube malformation (NTD). According to most studies, the detection rate (DR) for T21 is approximately 70 - 80% in the first trimester and 50 - 70% in the second trimester but is accompanied by a high false positive rate (FPR) [3]. This method does not directly detect the fetal chromosome copy numbers, and factors such as gestational age and NT have a great influence; the positive predictive value (PPV) is relatively low. With regard to health economics, prenatal serological screening is still the first-tier screening method for the prevention of chromosome diseases [4].

Since 2010, the noninvasive prenatal test (NIPT) based on free fetal DNA fragments in maternal peripheral blood has been rapidly applied in clinical practice [5]. This technology uses next generation sequencing (NGS) to obtain cell-free DNA fragments (cfDNA) from each chromosome and finally determines the risk of fetal aneuploidy of chromosome 21, 18, and 13 through bioinformatic analysis. This technology has a high PPV and a low false negative rate. The widespread application of NIPT has greatly reduced the number of live births of T21 in recent years. However, this method also has inherent defects such as being easily affected by maternal background DNA and difficulty in avoiding confined placental mosaicism (CPM) [6].

In this study, we conducted a retrospective study to assess missed T21 fetuses in prenatal serological screening and to explore how to reduce missed detection of T21 fetuses in clinical practice.

MATERIALS AND METHODS

Study population

Prenatal serological screening and NIPT are both free in some cities in China. Pregnant women voluntarily choose, and doctors provide genetic counseling for abnormal results. All of the 116 pregnant women with trisomy 21 fetuses confirmed by karyotype analysis or chromosome microarray analysis (CMA) from July 2012 to June 2022 were selected as study subjects; 60 were screened in the first trimester, 46 were screened in the second trimester, and 10 were screened in the first and second trimester. Data from serological screening and NIPT were analyzed. The 116 subjects were divided into high-risk, moderate-risk, and low-risk groups according to the results of the serological screening. The study was approved by the Ethics Committee of the Longgang District Maternity & Child Healthcare Hospital of Shenzhen City before implementation. All subjects provided written informed consent to conduct scientific research on their data anonymously.

Prenatal serological screening

A 2 mL sample of non-anticoagulant blood from pregnant women was collected and naturally coagulated. Next, 0.8 - 1 mL of the serum was separated and repackaged by centrifugation at 3,500 x g within 2 hours for 5 minutes. The serum was stored at 4℃ before detection. The pregnancy assessment is based on the priority order of IVF > ultrasound information > last menstrual cycle. The detection system used was an AutoDELFIA1235 fully automatic time-resolved fluorescence immunoassay analyzer (PerkinElmer, Finland). In early pregnancy (9 + 0 to 13 + 6 weeks), free-HCGβ and PAPP-A were tested to calculate the risk of developing T21 and T18 in fetuses, combined with information such as NT, which should be performed by a sonographer with a good experience in the field, maternal age, maternal weight, and gestational age. During mid pregnancy (15 + 0 to 20 + 6 weeks), AFP, free-HCGβ, and uE3 were measured to calculate the risk of developing T21, T18, and NTD in fetuses based on information such as maternal age, gestational age, and maternal weight. The high-risk cutoff value for T21 was 1:270 and the moderate-risk cutoff value was 1:270 - 1:1,000. If the case was not considered a high-risk case, it was defined as a missed case.

NIPT

For each participant, 5 mL of maternal whole blood was collected by using EDTA-K2 tubes (BD, UK). Cell-free maternal plasma was separated and purified by centrifugation at 1,600 x g (10 minutes, 4℃) for whole blood and 13,000 x g (10 minutes, 4℃) for plasma, sequentially. NIPT specimens were stored at -80℃ for no more than 3 days before detection. DNA extraction and library construction were performed for each sample, following the manufacturer's instructions for the NIPT chromosomal abnormality test kit (BGI, Wuhan, China). Library qualification was determined by using Qubit 3.0 (Thermo, USA). A total of 48 libraries were pooled into one mixed library, which was single-end sequenced on the BGI SEQ500 platform (BGI, Wuhan, China) with 35-read length and 9.7 M average reads per sample. Bioinformatic analysis was performed on the BGI Halos platform, and a Z value > 3 indicated high risk. The cutoff value for the low fetal fraction of cfDNA was 3.5%.

Data analysis

The results of prenatal serological screening and NIPT were compared for each case to analyze the potential for complementary clinical applications of the two technologies.

For false negative results in prenatal serological screening in the first trimester, the risk values of T21 were recalculated after removing the NT measurement. Excel spreadsheets and R-4.1.0 were used to analyze the statistical differences of PAPP-A, AFP, uE3, and free-HCGβ between high- and non-high-risk cases.

The gestational diseases of the 116 pregnant women

Table 1. Characteristics of the study subjects.

Clinical characteristics	First trimester		Second trimester		Total
	High-risk	Non-high-risk	High-risk	Non-high-risk	
Samples	53	17	39	17	126 *
Expected age (years)	34.4 [30.3 - 38.2]	32.8 [30.1 - 33.8]	34.4 [29.4 - 37.4]	30.1 [28.2 - 35.5]	33.74 [29.02, 37.09]
Maternal weight (kg)	55.0 [51.0 - 64.0]	54.0 [51.0 - 60.0]	52.7 [50.9 - 59.7]	54.4 [50.0 - 56.5]	54.4 [50.78, 61.0]
Gestational week (days)	89 [85 - 91]	88 [88 - 92]	120 [115 - 129.5]	121 [116 - 129]	94 [88, 119.75]
T21 serological screening risk value					
High-risk	92				92
Moderate-risk	24				24
Low-risk	10				10

* 10 cases participated in the first and second trimester screening.

Table 2. Comparative analysis of clinical indicators between high-risk and non-high-risk results.

Factors	First trimester	Second trimester	p *	
			First trimester	Second trimester
PAPP-A-corrected MOM value				
Non-high-risk	0.6 [0.49 - 0.87]	/	0.12000	/
High-risk	0.44 [0.32 - 0.62]			
NT-corrected MOM value				
Non-high-risk	1.13 [1.04 - 1.38]	/	0.00460	/
High-risk	1.59 [1.28 - 1.955]			
hCG β-corrected MOM value				
Non-high-risk	1.28 [0.883 - 2.29]	1.84 [1.47 - 2.14]	0.00870	0.00190
High-risk	2.88 [1.717 - 3.878]	3.83 [2.375 - 5.52]		
AFP-corrected MOM value				
Non-high-risk	/	1.04 [0.85 - 1.23]	/	0.01700
High-risk		0.71 [0.605 - 0.955]		
uE3-corrected MOM value				
Non-high-risk	/	0.78 [0.68 - 0.87]	/	0.93000
High-risk		0.77 [0.645 - 0.89]		

* - Wilcoxon method.

were analyzed to determine whether they had an impact on the results of the serological screening.

RESULTS

Participant profiles

In this retrospective study, we analyzed 116 pregnant women diagnosed with T21 fetus during pregnancy. The profiles of expected age, maternal weight, gestational week, and serological screening risk value for T21 are shown in Table 1. There were no significant differences in terms of expected age, gestational week, and maternal weight between the missed and high-risk pregnant women.

Serological screening and NIPT results

Out of all 116 cases, 60 (51.72%) fetuses were screened only in the first trimester, 46 (39.66%) fetuses were screened only in the second trimester, and 10 (8.62%)

fetuses were screened in the first and second trimester. Seventeen T21 fetuses were missed in the first or second trimester screening, of which 5 T21 fetuses were missed in the first and second trimester screening with moderate or low risk. The expected age, maternal weight, corrected MOM value, and T21 risk value for all missed cases are shown in Table S1. Six pregnant women with an expected age > 35 years were considered older aged mothers. Ten of the 34 results showed abnormal PAPP-A, AFP, uE3, or free-HCGβ, of which 9 were of moderate risk for T21 and 1 was of low risk. In total, 48 out of the 116 pregnant women underwent NIPT in the second trimester and all showed high risk for T21. The proportion of NIPT detection was 41.38%. The NIPT outcomes of the 13 missed T21 in serum screening are shown in Table S1.

Data reanalysis

Among the 17 missed cases of T21 in the serological screening of the first trimester, 11 were reanalyzed after removing the NT values, 3 cases changed from moderate to high risk, 2 cases changed from low to moderate risk, 1 case changed from moderate to low risk, 3 cases remained unchanged in moderate risk, and 2 cases remained unchanged in low risk (Table S1). The NT value was high in the T21 risk calculation.

Comparative analysis of serum indicators between missed T21 and detected T21 revealed significant differences in NT, free-HCGβ, and AFP values (Table 2). NT and free-HCGβ values of the detected T21 cases were higher than those of the missed T21 cases, while the AFP levels were lower.

Pregnancy disorders were retrieved from the hospital medical records; no special disease diagnosis or medication history was found for those pregnant women.

DISCUSSION

Since its implementation in clinical practice, serological screening has been considered an economic, rapid, and highly sensitive prenatal screening method for T21. However, there are some deficiencies that limit its screening efficacy [7-10]. First, the target molecules for detection are PAPP-A, AFP, uE3, and free-HCGβ, which reflect the function and development of the placenta and are affected by multiple maternal factors. Second, the technology requires a high accuracy of information from the pregnant women such as gestational week, maternal weight, and age; inaccurate information will significantly bias the results. Third, from the perspective of the algorithm used, the weight of NT in the first trimester is high; thus, the accuracy of NT measurement has a great impact on the outcome of risk. Finally, FPR is high and PPV is low, leading to a decrease in compliance of pregnant women with prenatal diagnosis and an increased burden of anxiety. Following the local screening strategy, in the first and second trimester, free-HCGβ+PAPP-A+NT triple screening and free-HCGβ+AFP+uE3 triple screening were used, respectively. Data analyses from our laboratory of the past three years have shown that the high-risk rate of T21 in the first trimester was 3.69%, the FPR was 2.72%, the DR was 79.2%, and the PPV was 1.86%. In the second trimester, the high-risk rate of T21 was 3.09%, the FPR was 3.0%, the DR was 66.7%, and the PPV was 0.53%. This study analyzed the prenatal serological screening outcomes of 116 pregnant women with T21 fetus. The average age of the 116 pregnant women was 33.39 years, with a maximum of 44.13 years and a minimum of 20.45 years; 57.76% were under 35 years old. A total of 29 cases were missed in serological screening, of which 5 cases were not high-risk in both the first and the second trimesters screening, 23 cases were of moderate risk, and 11 cases were of low risk. The missed diagnosis rate was 25% (29/116). Among the missed cases, 67.65% (23/34) were of moderate risk. The moderate risk of serological detection should receive closer attention in clinical practice. Prenatal diagnosis or other methods with higher detection rates should be implemented. Furthermore, among the 29 missed cases, 79.31% (23/29) of the pregnant women were under 35 years of age, which indicates that prenatal screening should not be ignored in pregnant women under 35 years of age.

NIPT can detect fetal trisomy of chromosomes 13,18, and 21 and reveal monogenic genetic disease, microdeletion, and microduplication through high-throughput sequencing of cfDNA in maternal plasma [11,12]. Although compared to traditional serological screening, NIPT cannot detect NTD, but according to previous studies, the sensitivity of NIPT to T21, T18, and T13 is more than 98% and the specificity is more than 99% [13]. Data analysis from our laboratory in the past three years has shown that the DR of NIPT for T21 is 98.85%, the PPV is 83.49%, and the negative predictive value is 99.99%; thus, it is an efficient screening method for T21. However, it should be emphasized that NIPT is not a diagnosis, but only a screening method. If the NIPT result indicates high risk, the pregnancy cannot be terminated directly without prenatal diagnosis. Amniocentesis and karyotype analysis or other genetic analysis should be performed. In this study, 116 pregnant women diagnosed with T21 fetuses were collected from July 2012 to June 2022, and the NIPT detection rate was 41.38%. In the past three years, the NIPT screening rates for pregnant women in our hospital were 65.67%, 67.8%, and 85.55% respectively, which shows an increasing trend.

Fetal NT is an ultrasound finding indicating the physiological accumulation of fluid under the skin behind the fetal neck in the first trimester. In the early 1990s, the relation between NT widening and chromosome abnormality was first discovered and reported by Nicaolades et al. [14]. Since then, NT has been considered the most specific and sensitive ultrasound indicator to predict fetal chromosome abnormalities. For example, Christiansen et al. studied the distribution of NT values on differ-

ent abnormal chromosomes and found that the median NT of T21 fetuses was 2.8 mm and that there was a significant difference compared to normal fetuses [15]. However, it is important to note that the measurement of NT of the fetus requires the mid-sagittal position, which has high requirements for the position of the fetus and the skills of the ultrasound doctors. Inaccurate NT values may affect serological screening results of the first trimester. In this study, the serological screening risk value in the first trimester of 11 pregnant women was reanalyzed after removal of NT, and it was found that 7 cases changed to a higher risk value and 4 changed to a lower risk value, of which 3 cases switched from moderate risk to high risk and 2 cases switched from low risk to moderate risk. When the NT value was < 2 mm, the risk value recalculated after removing NT increased. When the NT value was > 2 mm, the risk value recalculated after removing the NT decreased. This further explains the high weight of NT and the impact of NT accuracy in serological screening in the first trimester. For NT in the first trimester, only measurements from doctors with training and prenatal diagnosis qualification should be accepted.

Furthermore, this study compared the differences between the clinical indicators of missed T21 and detected T21 fetuses and found that there were statistical differences between the two groups in terms of NT, free-HCGβ, and AFP levels. The NT and free-HCGβ of detected T21 cases were higher than those of missed T21 cases, while the AFP values were lower. HCGβ is a glycoprotein secreted by placental trophoblast cells. It was originally proposed by Bogart et al. in 1987 and recommended to be applied to the prenatal screening of T21 [16]. Wald et al. found that the placental development of T21 fetuses was relatively slow, the level of HCGβ also decreased more slowly, resulting in a higher level than the average of normal fetuses at the same gestational age [17]. AFP is a globulin synthesized in the fetal liver and mainly reflects the development of fetal liver function. In the second trimester of pregnancy, the level of AFP in the maternal blood of normal pregnancy increases with gestational age, but in combination with the T21 pregnancy, the AFP value of maternal blood is lower than that of normal pregnancy due to fetal liver dysplasia and fetal maturity occurring later than a normal fetus [18]. When serological screening shows low risk, but NT, free-HCGβ, or AFP is abnormal, greater clinical attention should be paid. This study analyzed the impact of pregnancy disorders on serological screening results, but unfortunately, no specific pregnancy complications were found in missed T21 pregnant women.

In summary, we analyzed the prenatal serological screening and clinical test data of 116 pregnant women with T21 fetus. Prenatal screening was also found to be important for young pregnant women; NT measurement should be standardized, and the interpretation of NT should be cautious. In addition to risk value, analysis of abnormal single serum markers is also important; pregnant women with moderate risk or abnormal single serum markers can use NIPT for further evaluation, and NIPT can be extended to first-tier screening applications under the provision of economic support.

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Declaration of Interest:

The authors report that they have no conflicts of interest regarding this work.

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