

Second Trimester Prenatal Screening for Down's Syndrome in Mainland Chinese Subjects using Double-Marker Analysis of α -fetoprotein and β -human Chorionic Gonadotropin Combined with Measurement of Nuchal Fold Thickness

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Abstract

Introduction: This study examines the effectiveness of double-marker analysis for α -fetoprotein (AFP) and β -human chorionic gonadotropin (β -hCG) combined with measurement of nuchal fold thickness (NT) in the detection of Down's syndrome (DS) in Mainland Chinese subjects during second trimester prenatal screening. **Materials and Methods:** We examined pregnant women with a singleton pregnancy between 15 and 21 weeks of gestation who underwent second trimester screening for DS using double-marker analysis for AFP and β -hCG combined with ultrasound measurement of NT. The combined risk of DS was calculated. A cut-off of 1/270 was used to define a pregnancy at high-risk of DS. Amniocentesis was offered to all patients with high-risk pregnancies. **Results:** Using double-marker analysis for AFP and β -hCG in combination with measurement of NT, the detection rate of DS increased from 66.7% to 77.8% when compared with double-marker analysis alone with similar false-positive rates (4.35%, 4.83% respectively). Using receiver operating characteristic curve (ROC) analysis, we determined that the double-marker analysis combined with measurement of NT exhibited an increased area under the curve (AUC) of 0.835 (95% CI: 0.743 to 0.927) when compared to double-marker analysis alone, which had an AUC of 0.748 (95% CI: 0.635 to 0.860). In addition, both methods were more effective than any other single test such as AFP, free β -hCG or NT measurement. **Conclusion:** Second trimester prenatal screening using double-marker analysis for AFP and β -hCG combined with measurement of NT is effective for the detection of DS in Mainland Chinese pregnancies.

Ann Acad Med Singapore 2011;40:315-8

Key words: Down's syndrome, Prenatal screening, Second trimester

Introduction

Down's syndrome (DS) is the most common chromosome abnormality in mankind. Previously, patients underwent invasive prenatal diagnostic tests, such as chorionic villus sampling and amniocentesis, to detect chromosomal abnormalities. However, these invasive procedures have an intrinsic risk of foetal loss.¹⁻³ In Mainland China, second trimester double-marker analysis remains the most common approach to assess a foetus' risk for DS during the second trimester, levels of α -fetoprotein (AFP) and free β -human chorionic gonadotropin (β -hCG) are used to refine estimates of risk based on maternal age. As a routine double-marker screen, this test can identify approximately 60% of foetuses

with DS, with a false-positive rate (FPR) of 3% to 5.0%.⁴⁻⁶ Due to the high rate of false negative results, the performance of the currently used second trimester screening methods need to be evaluated.⁷ The effectiveness of nuchal fold thickness (NT) as an ultrasonic screening tool for Down syndrome has been independently confirmed by 2 large prospective clinical trials.^{8,9}

Therefore, in this study, we assessed the clinical utility of double-marker analysis for AFP and free β -hCG combined with ultrasonic measurement of NT to evaluate its effectiveness in detecting of DS in second trimester foetuses in a large cohort of Mainland Chinese subjects.

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Materials and Methods

Subjects and Sample Collection

A total of 10,556 patients aged ranging from 21 to 40 years with a single pregnancy between 15 and 21 weeks of gestation who attended our hospital, were sequentially enrolled in the study between February 2007 and May 2010. All the patients provided informed consent. The local Ethics Committee of the School of the Harbin Medical University approved the study conducted by department of Clinical Laboratory, the Fourth Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China.

Maternal blood samples were collected (4 mL) and centrifuged at 4000×g for 15 min within 2 hours of collection. Part of the serum was used for second trimester prenatal screening, and the remainder was stored at −80°C.

Ultrasound Examination

All the subjects screened were offered ultrasound with NT measurement. A foetus with a NT level higher than 6 mm was considered to be at increased risk for DS.⁹ The gestational age of the foetus was determined using the biparietal diameter (BPD).

Serum Analysis

The levels of AFP and free β-hCG were measured using Immunochemiluminescent methods (Access2, Beckman, UK). Multiples of medians (MoM) were automatically converted by the concentrations of the biochemical markers and corrected for maternal age, weight and self-recorded smoking status. The risk of DS was calculated using TCSOft. A cut-off of 1/270 was used to determine the risk of DS.¹⁰ Thus, a value of ≥1 in 270 was defined as indicating a high risk of DS, and patients in the high-risk group were offered amniocentesis. A value of less than 1/270 was considered as having low risk of DS, and patients in this group were followed-up until they gave birth. All the foetuses identified as being at high risk for DS were also evaluated by karyotype analysis.

Statistical Analysis

Statistical analysis was performed using SPSS 13.0 software. Statistical significance was evaluated by analysis of the 95% CI of the difference of independent proportions. *P* values of <0.05 were considered statistically significant. The chi-square test was used. The receiver operating characteristic (ROC) curves were analysed to determine the sensitivities of the detections.

Results

During the study period, a total of 10,556 pregnant subjects underwent the prenatal screen for DS, and 18 were found to be affected with DS. Upon screening for the 2 maternal serum markers, AFP and free β-hCG, 490 (4.65%) patients had a positive screening result, including 12 (2.45%) foetuses subsequently found to have DS. The DR for DS was 66.7%, with a FPR of 4.35% and a FNR of 33.33%.

When performing the double-marker analysis for AFP and free β-hCG in combination with the measurement of NT, 34 subjects deemed to be screening negative based on the analysis of the maternal serum subsequently tested screening positive by NT measurement, and 2 (5.88%) out of the 34 pregnancies were found to be affected with DS. We determined that the combined method had a DR of 77.8%, which increased by 11.1% (from 66.7% to 77.8%); a FPR of 4.83%, and a FNR of 22.22%. Table 1A shows the characteristics of the 18 patients with DS affected pregnancies who underwent double-marker analysis or double-marker analysis combined with measurement of NT.

We also analysed 238 pregnant subjects in the high-risk age group of above 35 years who underwent the prenatal screen for DS, and found that 9 (3.78%) were affected with DS. When performing the double-marker analysis combined with the measurement of NT, the DR for DS was 88.89% with a FNR of 11.11%. Table 1B shows the characteristics of 238 pregnant subjects who underwent double-marker analysis or double-marker analysis combined with measurement of NT in the high-risk age group above 35 years old.

ROC curves were generated with the data obtained from the DS-affected and unaffected pregnancies to evaluate the characters of the serum markers according to the median

Table 1A. Data of The Patients with DS-affected Pregnancies

	MSM* (n = 12)	(MSM + NT)† (n = 14)	<i>P</i> value
DR	66.7%	77.8%	<i>P</i> <0.05
FPR	4.35%	4.83%	NS
FNR	33.33%	22.22%	<i>P</i> <0.05

*double-marker analysis, DS affected

†double-marker analysis combined with measurement of NT, DS-affected

n: number of pregnancies; DR: detection rate; FPR: false-positive rate; FNR: false-negative rate; NT: nuchal fold thickness

Table 1B. Data of the High-risk Age Group Pregnancies of above 35 years

	MSM* (n = 7)	MSM + NT† (n = 8)	P value
DR	77.78%	88.89%	$P < 0.05$
FPR	7.98%	8.4%	NS
FNR	22.22%	11.11%	$P < 0.05$

*double-marker analysis, DS affected; †double-marker analysis combined with measurement of NT, DS affected

n: number of pregnancies; DR: detection rate; FPR: false-positive rate; FNR: false-negative rate; NT: nuchal fold thickness; NS: not significant, $P > 0.05$; MSM: maternal serum markers

MoM and median NT measurement. Figure 1A shows the comparison of the individual ROC curves for AFP, free β -hCG and NT. The results showed that the individual AFP, free β -hCG and NT tests had AUC values of 0.635 (95% CI, 0.515 to 0.755), 0.730 (95% CI, 0.512 to 0.949) and 0.714 (95% CI, 0.601 to 0.826), respectively. However, the double-marker analysis combined with NT had an AUC of 0.835 (95% CI, 0.743 to 0.927), which was significantly higher when compared to the double-marker analysis alone (AUC: 0.748; 95% CI, 0.635 to 0.860). Thus, the AUC of the double-marker analysis combined with the measurement of NT increased by 8.7% (from 74.8% to 83.5%) when compared to the double-marker analysis alone. Figure 1B shows ROC curves for the high-risk age group >35 years, the individual AFP, free β -hCG and NT tests had AUC values of 0.680 (95% CI, 0.504 to 0.855), 0.816 (95% CI, 0.676 to 0.955) and 0.778 (95% CI, 0.629 to 0.927), respectively. Double-marker analysis and the double-marker analysis combined with NT had AUC values of 0.839 (95% CI, 0.710 to 0.928) and 0.898 (95% CI, 0.763 to 0.972).

Discussion

Second trimester prenatal screening for foetal DS is well established in many Western countries⁵ and measurement of the maternal serum levels of AFP, free β -hCG and uE3 is used as a routine triple-marker screen.^{11,12} However, as Mainland China is a developing country, the policies for prenatal screening should be adjusted according to the economy of an individual country.¹³ Currently, the first trimester screening for DS that involves the use of ultrasound for the assessment of nuchal translucency thickness is not available in Mainland China.⁴ At present, some institutions conduct second trimester prenatal screening for DS by performing maternal serum triple-marker analysis or contingent triple screening in China.¹³ However, due to the current medical conditions in Mainland China, this triple-marker screening method for the detection of DS is neither an attractive nor cost-effective strategy.¹⁴ As such, pregnant women in Mainland China typically undergo double-marker screening

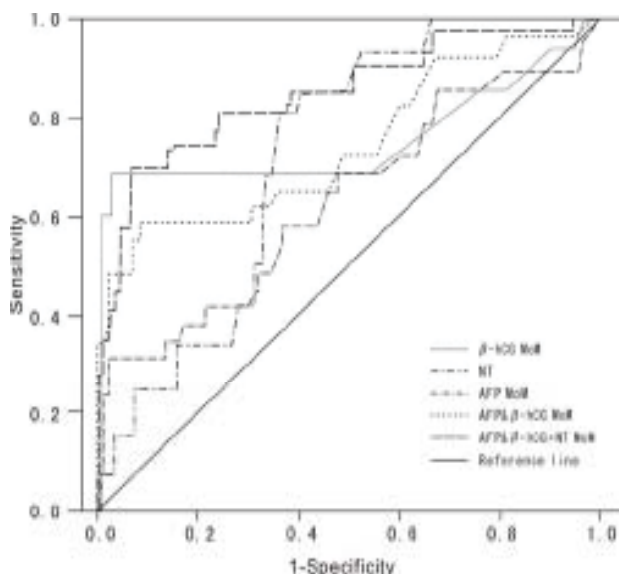


Fig. 1A. Receiver operating characteristic (ROC) curves for DS affected and unaffected pregnancies based on the median MoM and median NT. The SEs of AFP, free β -hCG, NT, double-marker analysis (AFP and free β -hCG), and double-marker analysis combined with NT were 0.061, 0.112, 0.057, 0.057 and 0.047, respectively.

MoM: multiples of medians; AFP: α -fetoprotein; NT: nuchal fold thickness

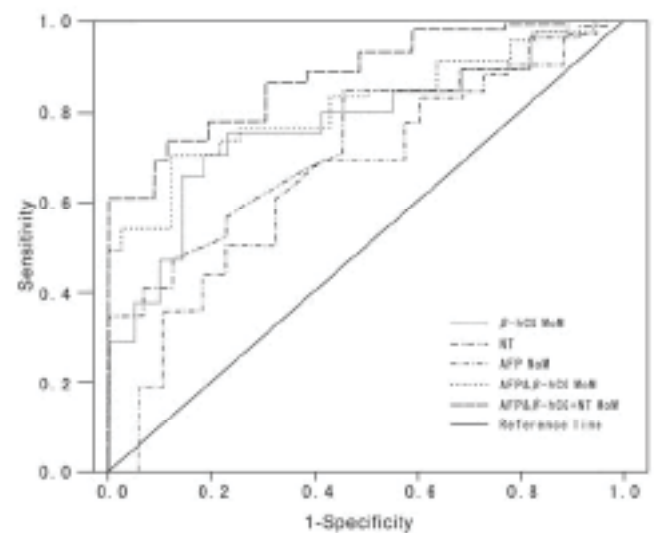


Fig. 1B. ROC curves for the high-risk >35 years age group of DS affected and unaffected pregnancies based on the median MoM and median NT. The SEs of AFP, free β -hCG, NT, double-marker analysis (AFP and free β -hCG), and double-marker analysis combined with NT were 0.057, 0.071, 0.076, 0.066 and 0.051, respectively.

MoM: multiples of medians; AFP: α -fetoprotein; NT: nuchal fold thickness

for AFP and free β -hCG because of its lower cost.

Ultrasonic measurement of NT has recently been proposed to be a useful parameter for the detection of fetal chromosomal abnormalities, such as DS.¹⁵ The use of an NT >6 mm has been reported in numerous studies based on foetal DS scan data usually between 15 and 21 weeks gestation.¹⁶ For the first time in Mainland, China, we examined pregnancies between 15 and 21 weeks of gestation and performed second trimester prenatal screening for DS on the basis of a double-marker analysis combined with NT measurement.

Based on double-marker analysis of the serum, 6 DS affected pregnancies were missed; however, when the measurement of NT was included in the analysis, only 4 DS (14 out of 18) were missed, correlating with a 11.1% increase in the DR, and an FPR of 4.83%, not significantly different when compared to maternal serum screening of 4.35% (Table 1A).

A study in Taiwanese subjects showed that second trimester screening of AFP and free β -hCG in maternal serum has a DR of 56.5% and a FPR of 5.3%.¹⁷ A similar study in Hong Kong showed that this screening method has a DR of 57% and a FPR of 3%.⁵ Our results showing the efficiency of double-marker screening are in agreement with these reports and with another study performed in Mainland Chinese subjects.⁴

Using the ROC curves, we determined that measurement of NT alone is a less efficient screening test for DS compared to when it is combined with double-marker analysis.

We found that when the same approach was used for women >35 years age group, the DR was higher and FPR higher when compared to women of all ages.

Potential limitation of this study is the inclusion of mainly DS high-risk pregnancies. Other foetal chromosomal abnormalities and the values of NT also increased to varying degrees in second trimester. There is normal karyotype but the foetal heart malformations also appear with thickened NT. Therefore, further research is needed for NT in second trimester.

Conclusion

In conclusion, second trimester prenatal screening using double-marker analysis in combination with measurement of NT is effective for the detection of foetal DS in Mainland Chinese subjects. This method may be useful in other developing countries to screen for DS prenatally.

Acknowledgements

We thank the Department of Clinical Laboratory in the Fourth Affiliated Hospital of Harbin Medical University for their co-operation and support.

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