Original Article

Validity of Different Methods to Prenatal Screening for Down's Syndrom During First and Second Trimester Pregnancy of Chinese Women*

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Abstract

Objective To identify and determine the optimal method to screening for fetal Down's syndrome (DS).

Methods Three large cohorts with 17 118, 39 903, 16 646 subjects were enrolled for the first trimester double marker (pregnancy-associated plasma protein A and free β-human chorionic gonadotropin) screening (FTDMS), second trimester double marker (α-fetoprotein and free β-human chorionic gonadotropin) screening (STDMS), and second trimester triple marker (α-fetoprotein, free β-human chorionic gonadotropin and unconjugated estriol 3) screening (STTMS), respectively. The sensitivity, specificity, false positive rate (FPR), false negative rate (FNR) and the areas under ROC curves (AUCs) were estimated in order to determine the optimal screening method in women under or above 35 years old.

Results For women under 35 years old, STTMS was the best method with a detection rate of 68.8% and FPR of 4.3% followed by the STDMS with a detection rate (sensitivity) of 66.7% and FPR of 4.9%. The FTDMS had a lower detection rate of 61.1% and FPR of 6.3%. For women above 35 years old, the detection rate of all the methods was similar, but STTMS method had a lowest FPR of 15.9%. For women under 35 years old AUCs were 0.77 (95% CI, 0.64 to 0.91), 0.81 (95% CI, 0.71 to 0.91), and 0.82 (95% CI, 0.69 to 0.96) for FTDMS, STDMS, and STTMS methods, respectively; for those above 35 years old, AUCs were 0.70 (95% CI, 0.56 to 0.83), 0.70 (95% CI, 0.59 to 0.82), 0.78 (95% CI, 0.58 to 0.97) for FTDMS, STDMS and STTMS , respectively.

Conclusion Findings from our study revealed that STDMS is optimal for the detection of fetal DS in pregnant women aged under 35. For individual women, if economic condition permits, STTMS is the best choice, while for women aged above 35, STTMS is the best choice in this regard.

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

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INTRODUCTION

Down's syndrome (DS) is the most common chromosome abnormality in mankind. Previously, pregnant women usually underwent invasive prenatal diagnostic tests, such as chorionic villus sampling and amniocentesis in order to detect if there are chromosomal abnormalities. These invasive procedures pose an intrinsic risk of fetal loss[1-3].

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First-trimester double marker analysis which includes detecting levels of α-fetoprotein (AFP) and free β-human chorionic gonadotropin (β-hCG) screening for Down's syndrome by using ultrasonography to assess nuchal translucency has become widespread since its introduction by Nicolaides and his colleagues in the early 1990s[4-7]. And a study in US on the first-trimester screening to date, involving 8514 pregnancies, reported a 79 percent detection rate at a 5 percent false positive rate[8]. However, the second-trimester screening including determination of AFP and β-hCG remains the most common method for assessing the risk of DS in US.

In Mainland China, the first trimester double-marker analysis including pregnancy-associated plasma protein A (PAPPA) and free β-hCG screening, the second trimester double-marker analysis including AFP and free β-hCG screening, the second trimester triple marker analysis including AFP, free β-hCG and unconjugated estriol 3 (uE3) screening in combination with the first trimester double-marker and the second trimester triple marker screening have become the most common serum screening tests for DS. For all the screening tests, markers are used to refine estimate of risk based on maternal age, weight and gestation. The second trimester double-marker analysis including AFP and free β-hCG screening is a most commonly used method which can identify approximately 60% of fetuses with DS, and with a false-positive rate (FPR) of 3% to 5.0%[9-11]. Owing to the high rate of false negative results, this test used mostly with other available screening tests needs to be therefore evaluated[12].

In the present study, the trimester double-marker or triple marker analysis was used in order to evaluate its effectiveness in detecting DS in the first and the second trimester fetuses in three large subject cohorts in Mainland China. And as we all known, the effectiveness of chorionic villus sampling followed by karyotype analysis is the golden diagnostic standard for DS.

MATERIALS AND METHODS

Subjects and Sample Collection

All subjects were pregnant women who ever attended the clinic of the Maternal and Child Health Hospital of Hunan Province for prenatal screening. And most of them were living in Changsha City and its surrounding areas. This study included three large cohorts. One cohort including 17 118 women aged from 17 to 44 with a single pregnancy between 11 weeks and 13 weeks and 6 days of gestation were sequentially enrolled in the study for the first trimester double marker (PAPPA, β-hCG) screening (FTDMS) between July 2007 and December 2009. The second cohort including 39 903 women aged from 17 to 48 with a single pregnancy between 15 and 20 weeks of gestation were sequentially enrolled in the study for the second trimester double marker (AFP, β-hCG) screening (STDMS) between October 2004 and March 2010. And the third cohort including 16 646 women aged from 18 to 49 with a single pregnancy between 15 weeks and 20 weeks and 6 days of gestation were sequentially enrolled in the study for the second trimester triple marker (AFP, β-hCG, uE3) screening (STTMS) between July 2007 and March 2010. Informed consent was provided to all of the subjects enrolled in the study.

Maternal blood samples (4 mL) were collected and centrifuged at 4000 ×g for 15 min within 2 h of collection. Part of the serum was used for marker screening, and the remainder was stored at -80 °C for further analysis.

Serum Analysis and Risk Assessment

The levels of PAPPA, AFP, free β-hCG, and uE3 were measured using immunochemiluminescent methods (Access2, Beckman, UK). And multiples of medians (MoM) were automatically converted by the concentrations of the biochemical markers and corrected based on maternal age, weight, and gestation.

The risk on DS was calculated using Wallae 2T software for the subjects enrolled from October 2004 to September 2007 and, similarly, Lifecycle software was used for the subjects enrolled from October 2007 to March 2010. A cut-off of 1/270 was used to determine the risk on DS[13] and a value of ≥1 in 270 was therefore defined as indicating a high risk on DS, and women with positive results from either the first-trimester or the second-trimester screenings were offered formal genetic counseling and the option of amniocentesis for genetic analysis.

The women whose fetuses were identified through amniocentesis as being at high risk on DS were finally evaluated by karyotype analysis while the women whose fetuses were identified as being at high risk but did not accept amniocentesis, necessary followed-up was conducted until they gave birth at the end of their pregnancies. In order to determine whether the newborn baby was

affected with DS or not, karyotype analysis in which the DNA was drawn from the baby's serum should be performed.

A value of less than 1/270 was considered as having low risk on DS, and women in this group were followed-up until they gave birth and, whether their babies were affected with DS or not would also be assessed.

Statistical Analysis

As the prevalence of DS is much high in pregnant women aged above 35, the subjects of each cohort were therefore divided into two subgroups: pregnant women aged above 35 and pregnant women aged under 35. For each subgroup, the basic characteristic of the subjects were collected and analyzed and the numbers of babies affected with DS were calculated for both women with high or low risk on DS. In order to compare three screening methods and their detection rate (sensitivity), specificity, false positive rate (FPR), false negative rate (FNR), Youden index and areas under receiver operating curves (ROC) as well as the 95% confidence interval for each screening test were estimated . For the ROC analysis, the screening result (positive or negative by the cutoff value of 1/270) was used as the testing value, and the karyotype analysis was used as the golden standard as mentioned above. The statistical significance was defined as $P \leq 0.05$ and all statistical analyses were performed using SPSS 13.0.

RESULTS

The demographic characteristics of the 74 067 subjects enrolled are summarized in Table 1 and data on the age and weight of the women, and the length of their gestation were presented. The mean age of the women under 35 were 27.7, 27.6, and 27.4 for FTDMS, STDMS, and STTMS respectively, while the mean age of the women above 35 were 37.3, 37.2, and 37.3 for FTDMS, STDMS, and STTMS respectively. The mean length of gestation of the women aged under 35 was 11.9, 17.8, and 17.6 weeks for FTDMS, STDMS, and STTMS, respectively; and that of the women aged above 35 was 11.6, 17.7, and 17.7 weeks for FTDMS, STDMS, and STTMS respectively.

Table 1. The Demographic Characteristics of Individual Screening Tests for Subjects in Each Subgroup

Subgroup	Screening Method	No. of Subjects	Age[1] (yr)	Gestation[1] (week)	Weight[1] (kg)
<35 years	FTDMS	16104	27.7±3.2	11.9±1.4	52.9±7.5
	STDMS	38240	27.6±3.0	17.8±1.4	53.4±7.5
	STTMS	15661	27.4±3.2	17.6±1.5	54.9±7.5
≥35 years	FTDMS	1014	37.3±2.0	11.6±1.4	56.4±7.8
	STDMS	1663	37.2±2.0	17.7±1.5	58.2±8.3
	STTMS	985	37.3±2.0	17.7±1.5	58.8±8.0

Note. [1]mean±SD FTDMS: first trimester double marker screening; STDMS: second trimester double marker screening; STTMS: second trimester triple marker screening.

In Table 2, the results from the first- and second-trimester screening were summarized by counting the number of detected and false positive cases above the risk cutoff levels. And the positive rate of screening were 6.4%, 5.0%, and 4.3% for women aged under 35 for FTDMS, STDMS and STTMS respectively, while 32.8%, 22.6%, and 16.2% for those aged above 35 respectively.

In Table 3, the results from different screening methods for each subgroup of pregnant women were presented. For the women aged under 35, STTMS was the best method, with a detection rate of 68.8% and FPR of 4.3%. And the STDMS was the second, with a detection rate of 66.7% and FPR of 4.9%. FTDMS had a lower detection rate of 61.1% and FPR of 6.3%. For the women aged above 35, the detection rate of all three methods was similar, and the STTMS had a lowest FPR of 15.9%. The Youden index for the women aged under 35 were 54.8%, 61.7%, and 64.5% for FTDMS, STDMS, and STTMS respectively, while 39.1%, 41.0%, and 55.6% for the women aged above 35. The actual numbers by which the statistical indexes were calculated are presented in Appendix 1.

In order to compare the three different screening tests, we believe that the comparison should not be based on the point estimates of performance of the main screening tests, as shown in Table 3 and it should be however based on AUCs under ROC curves. Table 4 shows the estimated AUCs under ROC curves for three different screening methods for each subgroup of pregnant women. In the subgroup of women aged under 35, AUCs were 0.77 (95% CI, 0.64 to 0.91), 0.81 (95% CI, 0.71 to 0.91), and 0.82 (95% CI, 0.69 to 0.96) for FTDMS, STDMS and STTMS respectively while for women aged above 35, AUCs were 0.70 (95% CI, 0.56 to 0.83), 0.70 (95% CI, 0.59 to 0.82), and 0.78 (95% CI, 0.58 to 0.97) for FTDMS, STDMS, and STTMS, respectively. All the AUCs were significant at $P<0.05$, indicating that all the screening methods were efficient to certain extent and these were in consistent with the point estimation of the performance of the screening.

Table 2. Directly Observed Performance of Different Prenatal Screening Methods in Each Subgroup

Subgroup	Screening Method (*n*)	No. of Women with High Risk	Positive Rate of Screening(%)	No. of Fetuses with Down's Syndrome in High Risk Women	No. of Fetuses with Down's Syndrome in Low Risk Women
<35 years	FTDMS (16104)	1023	6.4	11	7
	STDMS (38240)	1902	5.0	20	10
	STTMS (15661)	676	4.3	11	5
≥35 years	FTDMS (1014)	333	32.8	10	4
	STDMS (1663)	376	22.6	15	6
	STTMS (985)	123	16.2	5	2

Table 3. Statistical Index Evaluation of Different Screening Methods in Each Subgroup

Subgroup	Screening Method	Sensitivity (%) 95% CI	Specificity (%) 95% CI	FPR (%) 95% CI	FNR (%) 95% CI	Youden index γ (%)
<35 years	FTDMS	61.1 (60.4,61.9)	93.7 (93.3,94.1)	6.3 (5.9,6.7)	38.9 (38.1,39.6)	54.8
	STDMS	66.7 (66.2,67.1)	95.1 (94.9,95.3)	4.9 (4.7,5.2)	33.3 (32.9,33.8)	61.7
	STTMS	68.8 (68.0,69.5)	95.8 (95.4,96.1)	4.3 (3.9,4.6)	31.3 (30.5,32.0)	64.5
≥35 years	FTDMS	71.4 (68.7,74.2)	67.7 (64.8,70.6)	32.3 (29.4,35.2)	28.6 (25.8,31.4)	39.1
	STDMS	71.4 (69.3,73.6)	69.6 (67.4,71.8)	30.4 (28.2,32.6)	28.6 (26.4,30.7)	41.0
	STTMS	71.4 (68.6,74.3)	84.2 (81.9,86.4)	15.9 (13.6,18.1)	28.6 (25.8,31.4)	55.6

Note. FPR: false positive rate; FNR: false negative rate.

Table 4. AUCs for Each Screening Method in Each Subgroup

Subgroup	Screening Method (*n*)	AUC[1]	*P*	AUC (95% CI)
<35 years	FTDMS (16 104)	0.77±0.07	0.000	(0.64, 0.91)
	STDMS (38 240)	0.81±0.05	0.000	(0.71, 0.91)
	STTMS (15 661)	0.82±0.07	0.000	(0.69, 0.96)
≥35 years	FTDMS (1014)	0.70±0.07	0.012	(0.56, 0.83)
	STDMS (1663)	0.70±0.06	0.002	(0.59, 0.82)
	STTMS (985)	0.78±0.10	0.011	(0.58, 0.97)

Note. [1]mean±SE.

DISCUSSION

In this study, the three different screening methods for risk on DS for pregnant women in Hunan Province in China was performed and compared for the optimization. And the results from the study showed that the second -trimester double marker screening for DS is highly effective, but adding uE3 as a third marker in the second -trimester screening yielded higher detection rates and lower false positive rates.

Then, we tried to adopt several steps in order to improve the quality control of the study. Firstly, the weight of pregnant women was measured at every day when blood sample was drawn because large sample studies have proved that the weight is negatively associated with MoM or marker concentration and all the risk values have therefore been adjusted for these women's weight. Secondly, for the pregnant women whose fetuses were identified as being at high risk on DS but refused to accept amniocentesis, the follow up via telephone inquiry was conducted until the time point they provided the outcome of the pregnancy so as to clarify whether the babies were affected with DS or not .

The second trimester prenatal screening for fetal DS is well established in many western countries and measurement of the maternal serum levels of APF, free β-hCG and uE3 is used in this method as a routine triple-marker screening tool[14-15]. Considering this however, the policies for prenatal screening seems need to be adjusted along with its economic development and social progress[16]. Currently, the first trimester screening for DS that involves the use of ultrasound for the assessment of nuchal translucency thickness is not widely used especially in middle-small cities in China[9] although this method is considered as a highly effective approach[17]. At the present, some institutions conduct the second trimester prenatal screening for DS by performing maternal serum triple-marker analysis or contingent triple screening in China[16]. And owing to the limitation of current medical conditions the triple-marker screening method for the detection of DS is neither an attractive nor a cost-effective strategy[18]. Pregnant women in Mainland China usually undergo double-marker screening for AFP and free β-hCG due to its relatively lower cost. And our study has also demonstrated that double-marker screening for AFP and free β-hCG in the second trimester could almost achieve the efficiency of triple screening with less cost.

A study in Taiwan showed that the second trimester screening of AFP and free β-hCG in maternal serum had a DR of 56.5% and a FPR of 5.3%[19]. A similar study in Hong Kong showed that this screening method had a DR of 57% and a FPR of 3%[10]. The results from our present study showed that the efficiency of double-marker screening are in agreement with those in Taiwan and in Hong Kong and, also, with that of another study conducted in Mainland China[9].

In our study, it was found that when the same method was used for pregnant women aged above 35, DR and FPR were both higher compared to those of women aged under 35. The Youden index and AUCs were low for each screening method in the subgroup due to the high FPR and this result has indicated that for the women aged above 35, STTMS should be considered as the first choice for prenatal screening and if possible, chorionic villus sampling and amniocentesis should be used instead of prenatal screening. Stepwise sequential screening, which combines the results of both the first-trimester and the second-trimester measurements with a final second-trimester risk assessment, in contrast, keeps false positive rate low and provides early results to women with a positive test. Unfortunately, though hundreds of the enrolled pregnant women in our study have joined in both of the first and second trimester screening, the dataset is not yet enough to assess this screening method. Further research is therefore needed in order to determine the most effective method of sequential screening by comparison with the findings from other screening programs.

CONCLUSION

In conclusion, the result from our study showed that the second trimester prenatal screening using double marker analysis is effective for the detection of fetal DS in pregnant women aged under 35. For individual woman, if economic condition permits, the second trimester prenatal screening using triple marker analysis is the best choice. For women aged above 35, the best choice of prenatal screening is the second trimester triple marker screening as well as chorionic villus sampling and amniocentesis instead of screening. And we believe that this method may be recommended to other developing

countries for the effective prevention of the birth of DS prenatally.

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Appendix 1

Table A1. Performance of FTDMS in Women under 35 Years Old

FTDMS	Karyotype Analysis		Total
	Positive	Negative	
Positive	11	1012	1023
Negative	7	15 074	15 081
Total	18	16 086	16 104

Note. FTDMS: first trimester double marker screening; STDMS: second trimester double marker screening; STTMS: second trimester triple marker screening.

Table A2. Performance of FTDMS in Women above 35 Years Old

FTDMS	Karyotype Analysis		Total
	Positive	Negative	
Positive	10	323	333
Negative	4	677	681
Total	14	1000	1014

Table A3. Performance of STDMS in Women under 35 Years Old

STDMS	Karyotype Analysis		Total
	Positive	Negative	
Positive	20	1882	1902
Negative	10	36 328	36 338
Total	30	38 210	38 240

Table A4. Performance of STDMS in Women above 35 Years Old

STDMS	Karyotype Analysis		Total
	Positive	Negative	
Positive	15	361	376
Negative	6	1281	1287
Total	21	1642	1663

Table A5. Performance of STTMS in Women under 35 Years Old

STTMS	Karyotype Analysis		Total
	Positive	Negative	
Positive	11	665	676
Negative	5	14 980	14 985
Total	16	15 645	15 661

Table A6. Performance of STTMS in Women above 35 Years Old

STTMS	Karyotype Analysis		Total
	Positive	Negative	
Positive	5	155	160
Negative	2	823	825
Total	7	978	985