

# SECOND-TRIMESTER MATERNAL SERUM QUADRUPLE TEST FOR DOWN SYNDROME SCREENING: A TAIWANESE POPULATION-BASED STUDY

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## SUMMARY

**Objective:** To assess the usefulness of quadruple test screening for Down syndrome in Taiwan.

**Materials and Methods:** Maternal serum concentrations of  $\alpha$ -fetoprotein, human chorionic gonadotropin, unconjugated estriol, and inhibin A were measured in 21,481 pregnant women from 15 to 20 weeks of gestation.

**Results:** Of the 21,481 women, 977 returned values greater than the high-risk cut-off value (1 in 270). Most of these women (86.2%) decided to have an invasive procedure for genetic diagnosis. Nine cases of Down syndrome and 19 cases of other chromosomal anomalies were detected prenatally. Two children with Down syndrome were diagnosed after delivery even though a low estimated risk was determined following the quadruple test. The detection rate was 81.8% (nine out of 11 cases), with a 4.4% false-positive rate. The median multiple of the median value for  $\alpha$ -fetoprotein, human chorionic gonadotropin, unconjugated estriol and inhibin A were 0.87, 2.34, 0.77 and 2.16, respectively, in affected cases.

**Conclusion:** This is the first study of the quadruple test for Down syndrome in a Chinese population. Our findings suggested that the second-trimester quadruple test provides an effective screening tool for Down syndrome in Taiwan. [*Taiwan J Obstet Gynecol* 2010;49(1):30–34]

**Key Words:** Down syndrome, quadruple test, second trimester

## Introduction

Second-trimester Down syndrome (DS) serum screening by maternal age and measurement of  $\alpha$ -fetoprotein (AFP) and human chorionic gonadotropin (hCG) has been an established practice in Taiwan since 1994 [1,2]. The average detection rate of this screening double test

is around 56%, with a 4.9% false-positive rate, which is compatible to that of Western countries [3]. The double test has had a huge impact in Taiwan, dramatically decreasing live births of children with DS during the last decade [4].

Only one tertiary center in Taiwan studied unconjugated estriol (uE3), in addition to hCG and AFP, as the third marker (triple test). Patient blood samples were stored in 1996 and uE3 levels analyzed in 2000. The overall DS detection rate of the triple test was as high as 78.6% [5]. Inhibin A is a heterodimeric glycoprotein mainly secreted from the corpus luteum and the placenta [6,7]. Previous studies have shown that second-trimester serum inhibin levels are raised in the serum of pregnant



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women carrying a fetus with DS [8,9]. Thus, the incorporation of inhibin A into maternal serum DS screening in the second trimester, along with AFP, hCG and uE3, was named the quadruple test, and was first used in 1996 [10]. The detection rate of the quadruple test is approximately 83%, which is comparable with the first-trimester combined test (nuchal translucency,  $\beta$ -hCG and pregnancy associated plasma protein A) [11].

According to the guidelines published by the American College of Obstetrics and Gynecologists, triple or quadruple testing should be offered to pregnant women when certificated doctors are not available to perform a first-trimester combined test [12]. The quadruple test began to be provided to the general population in Taiwan in January of 2008 [3]. The standard reference range for inhibin A has never been determined among Asian women, so a pilot study to determine normal values was performed for the purpose of establishing a database.

This is the first study describing the efficiency of using four DS markers for screening of a Chinese population. We evaluated the second-trimester quadruple test for DS in terms of simplicity, feasibility, and reliability among Taiwanese pregnant women.

## Materials and Methods

From July to December 2008, we conducted a population-based study of a second-trimester DS screening program. All of the women enrolled in the study were in their 15<sup>th</sup> to 20<sup>th</sup> week of gestation. These women were offered routine prenatal examinations in local hospitals or clinics where neither certified sonographers nor doctors were available to perform first-trimester nuchal translucency scanning. Since first-trimester combined testing was not possible, maternal serum testing was recommended and performed by local doctors. Only singleton and healthy pregnancies were enrolled in the study. The exclusion criteria included multiple pregnancies, maternal diabetes or cardiac disease, illiteracy (inability to read and sign the consent forms), and known high-risk pregnancies originally referred from the medical center. Ten milliliters of blood were taken from each eligible subject for study.

All basic pregnancy data, including age, body weight, delivery history, smoking history, assisted reproductive technologies used and ethnicity, were recorded. Every sample was collected and sent to a central laboratory in order to decrease the inter-operator error. The multiple of the medians (MoMs) for hCG, uE3, AFP and inhibin A were analyzed using the Access chemiluminescent enzyme-linked immunoassay system (Beckman

Coulter, Inc., Brea, CA, USA) with a built-in calibration system. The testing was performed according to the manufacturer's instructions.

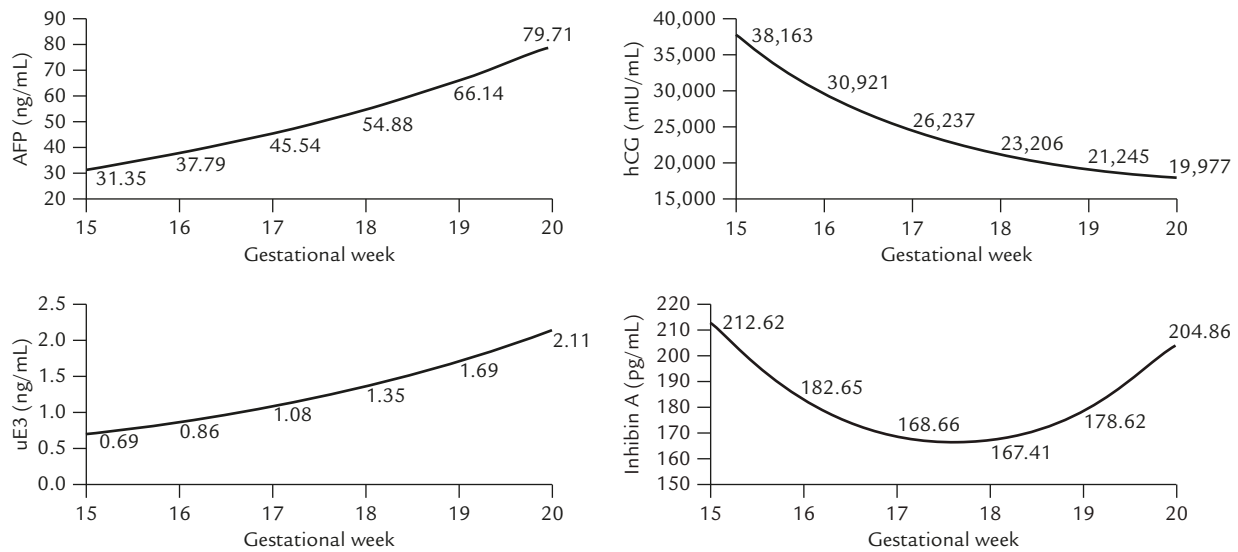
Risk calculation was performed using Benetech prenatal risk assessment software (Benetech Inc., Toronto, ON, Canada). Maternal age, body weight, ethnicity, cigarette smoking and the level of the four markers were factored in by the software. The high-risk cut-off level was defined as a risk greater than 1 in 270. Once high-risk women were identified, they were informed immediately via telephone either by their doctors or by well-trained nurses. The women were then given a counseling session regarding further invasive genetic testing, such as amniocentesis. High-risk patients could decide either to have amniocentesis at the local hospital or to receive a referral to a medical center. The final results of this examination were recorded in the central laboratory. Women at low risk for DS who had amniocentesis for any reason or live-born DS babies were also recorded.

Statistical analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) computer software. All the variables were checked for normal distributions using normal probability plots. Correlation between variables was computed using Pearson's correlation test. Differences between the normal population and affected fetuses were determined using the Mann-Whitney *U* test. Statistical significance was defined at  $p < 0.05$ .

## Results

There were 102,944 babies born from July to December 2008 in Taiwan. After exclusion of unsuitable subjects, 21,481 pregnant women were enrolled into this population-based study. The coverage rate was as high as 20.87%, meaning that over one-fifth of pregnant women underwent the second-trimester quadruple test. Mean maternal age and maternal body weight were  $29.5 \pm 3.6$  years and  $57.3 \pm 8.1$  kg, respectively.

The standard curves of inhibin A, hCG, AFP and uE3 were created from 372 normal pregnant women at 15 to 20 weeks gestation at the National Taiwan University Hospital. Log linear regression was applied to AFP and uE3. Exponential and log quadratic regression were used for hCG and inhibin A, respectively (Figure). Table 1 shows the distributions of the four serum markers by gestational week. The regression medians used to calculate MoMs of AFP, hCG, uE3 and inhibin A for each gestational age were generated from individual formulas provided by assay manufacturer. Thus, we uploaded our own Chinese data into the risk calculation software.



**Figure.** Standard curves of inhibin A, human chorionic gonadotropin (hCG),  $\alpha$ -fetoprotein (AFP) and unconjugated estriol (uE3) were created from 372 normal pregnant women at 15–20 weeks' gestation attending the obstetrics department at the National Taiwan University Hospital. Log linear regression was applied to AFP and uE3. Exponential and log quadratic regression were used for human chorionic gonadotropin and inhibin A, respectively.

**Table 1.** Regression of the four markers in weeks 15–20 of gestation

Week	n	AFP (ng/mL)		hCG (mIU/mL)		uE3 (ng/mL)		Inhibin A (pg/mL)	
		Observed	Regressed	Observed	Regressed	Observed	Regressed	Observed	Regressed
15	24	36.24	31.35	42,814	38,163	0.6404	0.69	242.30	212.62
16	87	43.01	37.79	32,064	30,921	0.8489	0.86	218.94	182.65
17	96	44.89	45.54	32,641	26,237	1.0310	1.08	190.44	168.66
18	104	56.75	54.88	25,496	23,206	1.2995	1.35	198.39	167.41
19	51	71.63	66.14	23,297	21,245	1.5546	1.69	198.30	178.62
20	10	97.93	79.71	20,450	19,977	2.4862	2.11	227.85	204.86

AFP =  $\alpha$ -fetoprotein; hCG = human chorionic gonadotropin; uE3 = unconjugated estriol.

The quadruple test revealed 977 high-risk pregnancies, with cut-off levels greater than 1 in 270. Of these 977 high-risk pregnancies, 840 women (86%) decided to have an invasive procedure for a genetic diagnosis. According to the reporting system, 28 chromosomal anomalies were detected, including nine DS, six sex chromosomal abnormalities, four unbalanced translocations, five cases of trisomy 18, and four cases of trisomy 13. During the 6-month study period, local doctors reported two children were born with DS, and that the initial risk assessments of DS indicated low risk in both cases. The detection rate was 81.8% (9/11) with a 4.4% false-positive rate.

Table 2 shows mean maternal age, body weight, gestational age and average MoM for each DS marker. Cases 1 to 9 were affected fetuses that were diagnosed prenatally by high-risk screening. All nine pregnancies were terminated. The MoMs of affected fetuses for AFP, hCG, uE3 and inhibin-A were 0.87, 2.34, 0.77 and 2.16, respectively. The differences in the MoM between the

normal population and the affected population differed significantly ( $p < 0.05$ ) for each serum marker. There was no difference in maternal age or maternal body weight (Table 3). Patients of Case 10 and 11 were born with DS despite low-risk results for the four-marker screening test. The overall risks for DS of these two cases were extremely low, without any ultra-high or ultra-low values for any single marker (Table 2).

## Discussion

We report the results of second-trimester quadruple screening for DS in a Chinese population. Since there was no available data for inhibin A among Asian women, we also established reference values for this marker and a calculation system to use before clinical testing. Our regression curves for the four markers were normally distributed. The most difficult part of the quadruple test was testing for inhibin A, as the regression line needed

**Table 2.** Down syndrome cases

Case	GA (wk)	Mat age (yr)	Weight (kg)	AFP (MoM)	hCG (MoM)	uE3 (MoM)	Inhibin-A (MoM)	Estimated risk	Outcome
1	17	25.5	91.6	0.55	2.34	0.59	2.54	1:6	Terminated
2	18	31.3	57.1	0.64	1.5	0.76	1.39	1:28	Terminated
3	15	27.0	53.2	1.27	2.46	0.71	5.48	1:60	Terminated
4	16	30.5	60.1	0.67	1.65	0.63	1.33	1:68	Terminated
5	16	34.0	66.3	0.73	4.24	1.07	2.50	1:39	Terminated
6	17	29.1	52.9	0.67	1.66	0.79	2.11	1:64	Terminated
7	16	29.4	51.6	1.31	3.00	0.63	1.26	1:197	Terminated
8	19	30.1	53.3	0.66	1.41	0.35	1.59	1:109	Terminated
9	18	35.2	48.2	0.65	4.86	0.77	3.13	1:6	Terminated
10	15	21.7	41.3	1.34	1.62	1.04	1.57	1:4,190	Live born
11	17	29.0	64.4	1.07	1.05	1.12	0.89	1:38,500	Live born
Mean	16.7	29.35	58.18	0.87	2.34	0.77	2.16		

GA = gestational age; Mat = maternal; AFP =  $\alpha$ -fetoprotein; MoM = multiple of the median; hCG = human chorionic gonadotropin; uE3 = unconjugated estriol.

**Table 3.** Comparison of unaffected and affected groups

	Unaffected	Affected	<i>p</i>
No. of sample	21,470	11	
Maternal age, mean (yr)	29.5	29.35	NS
Maternal weight, mean (kg)	57.29	58.18	NS
AFP (MoM)	1.05	0.87	<0.05
hCG (MoM)	0.97	2.34	<0.05
uE3 (MoM)	1	0.77	<0.05
Inhibin A (MoM)	1.04	2.16	<0.05

AFP =  $\alpha$ -fetoprotein; MoM = multiple of the median; hCG = human chorionic gonadotropin; uE3 = unconjugated estriol; NS = not significant.

more cases in the 15 or 20 weeks gestational age range to be complete. Our detection rate was 81.8%, which is comparable to most of the quadruple test studies in the world [13–16]. The false-positive rate was just under 5%, which led to fewer unnecessary invasive procedures than other screening strategies.

Only 11 DS cases were found among the 21,481 enrolled pregnancies, which was much lower than the incidence rate. This is mostly because all participants were recruited from local hospitals and clinics, not medical centers. When first-trimester combined testing was provided, couples usually elected early invasive genetic testing. When chromosomal anomalies were detected, these pregnancies were usually terminated at the parents' requests. Furthermore, for the pregnant population with advanced maternal age in Taiwan (older than 34 years), amniocentesis was accepted directly without any DS screening [17].

The screening strategy and clinical guidelines outlined by the Taiwan Society of Perinatology recommend

the first-trimester combined test as the method of choice [3]. There are only 40 to 50 qualified and certified obstetricians in Taiwan, which is far too few to meet the current demand. Quadruple maternal serum testing is fast becoming the second test of choice for DS screening in the United States and United Kingdom [12]. Because nuchal translucency scanning is not available in local hospitals, a reliable, easy, simple blood test is the best test methodology for both patients and doctors.

One of the limitations of this study was that reporting of high-risk pregnancies from local hospitals to the central laboratory was only 85.5% (data not shown). A comprehensive network needs to be established so that every woman who undergoes amniocentesis can be monitored. Secondly, the number of detected DS cases was small. Since a large population of pregnant women carrying fetuses with DS were detected early in their first trimester using the combined test in medical centers, obtaining blood samples from those mothers at 15 to 20 weeks gestation for positive controls would be highly beneficial to our study and the creation of database values. Finally, the data we collected was mostly from younger women. If the reference and testing samples incorporated data from older women, the findings may be much more convincing.

In conclusion, our preliminary study demonstrates DS detection rates with ideal false-positive rates in a Chinese population using the second-trimester quadruple test, comparable to that seen in Western countries. This test for DS screening in the Chinese population could be applied to routine prenatal checkups, especially when the clinical service is unable to provide certified and qualified ultrasonography personnel. A larger study with an older population is needed to determine cut-off values for that specific population.

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