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Original Article

First trimester combined test for Down syndrome screening in unselected pregnancies — A report of a 13-year experience

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Abstract

Objective: To analyze the performance of the first trimester Down syndrome screening in a single medical center in Northern Taiwan.
Materials and methods: From April 1999 to June 2012, a total of 25,104 pregnant women at gestational age of 10 weeks to 13 weeks 6 days received first trimester "combined test" for Down syndrome screening. The test combines the ultrasound scan of nuchal translucency thickness and maternal biochemical serum levels of pregnancy-associated plasma protein A (PAPP-A) and free beta-human chorionic gonadotropin (β-hCG). A positive screen was defined as an estimated Down syndrome risk ≥1/270, and either chorionic villous sampling or amniocentesis was performed for fetal chromosomal analyses.
Results: Seventy-eight of the 25,104 pregnancies were proven to have fetal chromosome anomalies. The detection rates for trisomy 21, trisomy 18, Turner syndrome, and other chromosome anomalies were 87.5% (21/24), 69.2% (9/13), 81.8% (9/11), and 60% (18/30), respectively, with a false positive rate (FPR) of 5.4% (1353/25,026). Further evaluation of the detection rates for trisomy 21, by gestational age at 11, 12, and 13 weeks, were 92.3%, 87.5%, and 66.7%, respectively.
Conclusion: The first trimester combined test is an effective screening tool for Down syndrome detection with an acceptable low false positive rate. The best timing of screening will be between 11 and 12 weeks' gestation.

Keywords: combined test; Down syndrome screening; first trimester; prenatal diagnosis

Introduction

In 1984, Merkatz et al found an association between second trimester low maternal serum level of alpha-fetoprotein (AFP) and fetal Down syndrome [1]. Later, the double test that added human chorionic gonadotropin (hCG) was introduced as a prenatal screening tool for Down syndrome in the second trimester, and this test has been a popular screening method in Taiwan since 1994. However, the detection rate for Down syndrome of the double test reported by Taiwan Down Syndrome Screening Group was approximately 56.5%, with a false positive rate (FPR) of 5.3% [2,3]. The first trimester combined test, which measures the thickness of nuchal translucency, and serologic tests, which measure pregnancy-associated plasma protein A (PAPP-A) and free beta-hCG (β-hCG) [4], have been proven as effective screening methods for Down syndrome, with a higher detection rate of 85–90% for an FPR of 5% [5–18]. Since April 1999, the first trimester combined test has been offered to every pregnant woman who came to Cathay General Hospital, Taipei, during 10–13 weeks of gestation. Recently, a second trimester quadruple test, that added unconjugated estriol (uE3) and inhibin A to the double test, has been developed for a higher

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detection of 80% with a FPR of 5% [19,20]. However, the quadruple test is reserved as an alternative test for pregnant women whose first prenatal visits are beyond 13 weeks and 6 days' gestation in the hospital. The aim of this study was to evaluate the performance of the first trimester combined test for Down syndrome detection.

Materials and methods

From April 1999 to June 2012, the first trimester combined test was routinely provided for pregnant women who came to Cathay General Hospital for prenatal care. The test involves measuring fetal nuchal translucency (NT) thickness and maternal serum levels of free β-hCG and PAPP-A at 10 weeks to 13 weeks 6 days of gestation. Fetal NT was measured according to the established criteria published by the fetal medicine foundation of the United Kingdom. The crown–rump lengths of fetuses were measured to determine the gestational ages. Maternal serum levels of free β-hCG and PAPP-A were determined simultaneously using a microtiter plate enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's protocol (Genemed Biotechnologies, South San Francisco, CA, USA). The values of NT, PAPP-A, and free β-hCG were divided by their respective day-specific median levels to determine the multiples of the median (MoM) of each marker. Down syndrome risk was calculated using Alpha Software (Logical Medical Systems, London, UK) based on a multivariate Gaussian distribution as described by Wald et al [21]. A positive screening result was defined as an estimated Down syndrome risk $\geq$1/270.

All chromosome analyses were carried out in the Prenatal Diagnosis Center of Cathay General Hospital in Taiwan. For cultured chorionic villous tissue and amniotic fluid cells, four primary cultures were performed using an *in situ* method with CHANG MEDIUM BMC (Irvine Scientific, Santa Ana, CA, USA). Microscopic analysis of Giemsa-stained chromosome banding used the rules for metaphase selection and colony definition as defined by Moertel et al [22]. The data of the combined test was extracted from a computerized Birth Database of Cathay General Hospital that was established by specially trained nurses in 1999. The research and ethics committee of Cathay General Hospital approved this study.

Results

Among the 25,104 pregnancies receiving the combined test during 10–13 weeks of gestation, there were 3326 (13%) at 10 weeks, 10,222 (41%) at 11 weeks, 8376 (33%) at 12 weeks, and 3180 (13%) at 13 weeks of gestation. The mean gestational age, maternal age, and maternal weight were 83 days, 29 years and 55 kg, respectively. There were 785 (3.1%) women >35 years of age and 304 (1.2%) women with multiple gestations. Among the multiple gestations, 302 women had twins and two had triplets. In total, 78 cases of chromosomal abnormality were identified. Among them, there were 24 cases of trisomy 21, 13 cases of trisomy 18, 11 cases of Turner syndrome, and 30 cases of chromosome anomalies including mosaicism, microdeletion, balanced and unbalanced chromosome structural anomalies, and other aneuploidy. With the cut-off value of $\geq$1/270, there were 1417 positive-screen pregnancies which occurred in 5.3% (1280/24,319) of women <35 years of age and 17.5% (137/785) of women of advanced maternal age. The detection rates for trisomy 21, trisomy 18, Turner syndrome, and other chromosome anomalies were 87.5% (21/24), 69.2% (9/13), 81.8% (9/11), and 60% (18/30), respectively (shown in Table 1). In trisomy 21, the MoM of maternal serum PAPP-A is 0.5 and free β-hCG is 1.8. In trisomy 18, the mean MoM of PAPP-A is 0.6 and free β-hCG is 0.7. In Turner syndrome, the mean MoM of PAPP-A is 0.5 and free β-hCG is 1.8. The detection rates for trisomy 21 at 11, 12, and 13 weeks were 92.3% (12/13), 87.5% (7/8), and 66.7% (2/3), respectively, with corresponding FPR values of 5.7%, 5.2%, and 3.9% (shown in Fig. 1).

Discussion

Two large clinical trials have demonstrated the effectiveness of first trimester combined test for Down syndrome screening. One was the SURUSS trial [14], including 47,053women primarily from the UK, which reported an 85% Down syndrome detection rate of the first trimester combined test under the FPR of 4.3%. The other one was the FASTER trial [5], including 38,000 women in the United States, which reported a similar result of an 85% detection rate under the FPR of 4.8%. This study showed that, with an FPR of 5.4%, the combined test could reach a Down syndrome detection rate of 87.5%, which was similar to the effectiveness of the two large series studies mentioned above.

The first trimester biochemical markers in this study showed that the mean values of free β-hCG and PAPP-A of 1.2 MoM (both in unaffected pregnancies) were higher than those which had been reported by the western countries [23,24], but were similar to a report of a predominantly Chinese population [9]. In trisomy 21 pregnancies, the mean MoM value decreased to 0.5

Table 1
Screening data of different fetal chromosome abnormalities.

	Trisomy 21 (n = 24)	Trisomy 18 (n = 13)	Turner syndrome (n = 11)	Others[a] (n = 30)
Mean NT thickness (MoM)	2.0	3.5	2.1	1.3
Mean PAPP-A (MoM)	0.5	0.6	0.5	0.9
Mean Free β-hCG (MoM)	1.8	0.7	1.8	1.3
Affected numbers	24	13	11	30
Detection rate (5% FPR)	87.5%	69.2%	81.8%	60%

β-hCG = beta human chorionic gonadotropin; FPR = false positive rate; NT = nuchal translucency; PAPP-A = pregnancy-associated plasma protein A.
[a] Others = other chromosome abnormality.

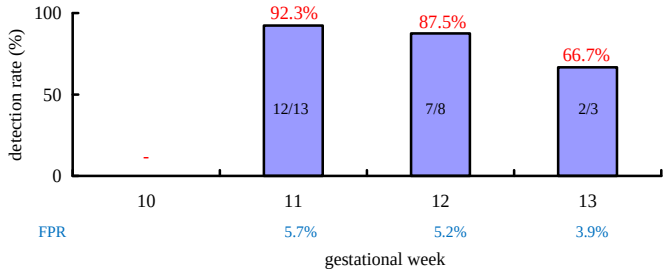

Fig. 1. The detection rate of Down syndrome by gestational week.

for PAPP-A, and increased to 1.8 for free β-hCG in our study population, which is consistent with what had been found in the Caucasian population (0.5 MoM for PAPP-A and 2.0 MoM for free β-hCG) [25]. Malone et al (2005) reported that the detection rates of first trimester Down syndrome screening at 11, 12, and 13 weeks were 87%, 85%, and 82%, respectively, with an FPR of 5% [5]. They proposed that the best timing to use the combined test is during 11–12 gestational weeks, because the difference of PAPP-A levels between trisomic and euploid pregnancies is greater than the difference of free β-hCG at this period of time. In this study, we also demonstrated that there was a higher detection rate (92.3%) at 11 gestational weeks, with an FPR of 5.7%. Therefore, we suggest that pregnant women should receive the first trimester combined test during 11–12 weeks of gestation.

Fetal NT is an effective ultrasound marker for Down syndrome screening. However, the effectiveness of the combined test mainly depends on the accuracy and quality of NT measurement. Previous publications indicated that NT thickness increases between 10 and 14 weeks of gestation due to physiological change. Pajkrt et al (1998) reported that NT thickness increased from 0.7 mm at 10 weeks of gestation to a peak of 1.7 mm at 13 weeks of gestation, and then declined to 1.0 mm at 14 weeks [26]. In this study, we found that the median NT thickness also increased with the increase of gestation week (0.97 mm for 10 weeks, 1.15 mm for 11 weeks, 1.35 mm for 12 weeks, and 1.55 mm for 13 weeks), which was consistent with the data above. The median MoM of NT was 1.04, thus we had a good quality control on fetal NT measurement.

The second trimester Down syndrome screening evolved from the previous double test to quadruple test, and is currently the mainstream test which can reach a higher detection rate of 75～80% with an FPR of 5% [5,19,20]. The test has been implemented in our hospital since 2008, and is provided to pregnant women whose first prenatal visit was beyond 13 gestational weeks or who have an intermediate risk (1/270～1/1000) in the combined test. At present, 3879 women received the quadruple test and the detection rate for Down syndrome was 83.3% (5/6) with an FPR of 6.3%. Although the second trimester quadruple test is not as effective as first trimester combined test, these two tests can be combined together as an integrated or sequential test. Both tests can yield a higher detection rate for Down syndrome to 95% with a 5% FPR and reduce the fetal loss rate associated with an invasive procedure [5].

Circulating cell-free fetal DNA from maternal plasma has offered a new approach for noninvasive prenatal diagnosis for fetal aneuploidies including trisomy 21, trisomy 18, trisomy 13, and sex chromosome numerous anomalies [27–29]. The Noninvasive Fetal Trisomy (NIFTY) test has yield 98.58～100% sensitivity and 97.95～100% specificity for Trisomy 21[30–33]. This test has two advantages. First, fetal DNA from maternal blood can be evaluated as early as 8 gestational weeks, which is earlier than the current first trimester Down syndrome screening. Second, there is no procedure related fetal loss associated with amniocentesis or chorionic villous sampling. However, not all healthy providers have the equipment and laboratory for the cell-free fetal DNA analysis, and the cost is much higher than Down syndrome screening, especially in Taiwan.

In conclusion, the first trimester combined test is an effective screening tool for Down syndrome detection, with an acceptable low false positive rate. The best timing of screening will be between 11 and 12 weeks' gestation.

References

[1] Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. Am J Obstet Gynecol 1984;148:886–94.
[2] Hsu JJ, Hsieh TT, Hsieh FJ. Down syndrome screening in an Asian population using alpha-fetoprotein and free beta-hCG: a report of the Taiwan Down Syndrome Screening Group. Obstet Gynecol 1996;87:943–7.
[3] Jou HJ, Shyu MK, Chen SM, Shih JC, Hsu JJ, Hsieh FJ. Maternal serum screening for Down syndrome by using alpha-fetoprotein and human chorionic gonadotropin in an Asian population. a prospective study. Fetal Diagn Ther 2000;15:108–11.
[4] Wald NJ, Hackshaw AK. Combining ultrasound and biochemistry in first-trimester screening for Down's syndrome. Prenat Diagn 1997;17:821–9.
[5] Malone FD, Canick JA, Ball RH, Nyberg DA, et al. First-trimester or second-trimester screening, or both, for Down's syndrome. N Engl J Med 2005;353:2001–11.
[6] Nicolaides KH. First-trimester screening for chromosomal abnormalities. Semin Perinatol 2005;29:190–4.
[7] Schielen PC, van Leeuwen-Spruijt M, Belmouden I, Elvers LH, Jonker M, Loeber JG. Multi-centre first-trimester screening for Down syndrome in the Netherlands in routine clinical practice. Prenat Diagn 2006;26:711–8.
[8] Scott F, Peters H, Bonifacio M, McLennan A, Boogert A, Kesby G, et al. Prospective evaluation of a first trimester screening program for Down syndrome and other chromosomal abnormalities using maternal age, nuchal translucency and biochemistry in an Australian population. Aust N Z J Obstet Gynaecol 2004;44:205–9.
[9] Leung TY, Chan LW, Leung TN, Fung TY, Sahota DS, Spencer K, et al. First-trimester combined screening for trisomy 21 in a predominantly Chinese population. Ultrasound Obstet Gynecol 2007;29:14–7.
[10] Jaques AM, Halliday JL, Francis I, Bonacquisto L, Forbes R, Cronin A, et al. Follow up and evaluation of the Victorian first-trimester combined screening programme for Down syndrome and trisomy 18. BJOG 2007;114:812–8.
[11] Schaelike M, Kossakiewicz M, Kossakiewicz A, Schild RL. Examination of a first-trimester Down syndrome screening concept on a mix of 11,107 high- and low-risk patients at a private center for prenatal medicine in Germany. Eur J Obstet Gynecol Reprod Biol 2009;144:140–5.
[12] Sahota DS, Leung TY, Chan LW, Law LW, Fung TY, Chen M, et al. Comparison of first-trimester contingent screening strategies for Down syndrome. Ultrasound Obstet Gynecol 2010;35:286–91.

[13] Wapner R, Thom E, Simpson JL, Pergament E, Silver R, Filkins K, et al. First-trimester screening for trisomies 21 and 18. N Engl J Med 2003;349:1405–13.
[14] Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). J Med Screen 2003;10:56–104.
[15] Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one-stop clinic: a review of three years prospective experience. BJOG 2003;110:281–6.
[16] Tsai MS, Huang YY, Hwa KY, Cheng CC, Lee FK. Combined measurement of fetal nuchal translucency, maternal serum free beta-hCG, and pregnancy-associated plasma protein A for first-trimester Down's syndrome screening. J Formos Med Assoc 2001;100:319–25.
[17] Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R, et al. First-trimester or second-trimester screening, or both, for Down's syndrome. N Engl J Med 2005;353:2001–11.
[18] Sahota DS, Leung WC, Chan WP, To WW, Lau ET, Leung TY. Prospective assessment of the Hong Kong Hospital Authority universal Down syndrome screening programme. Hong Kong Med J 2013;19:101–8.
[19] Wald NJ, Huttly WJ, Hackshaw AK. Antenatal screening for Down's syndrome with the quadruple test. Lancet 2003;361:835–6.
[20] Cuckle H, Benn P, Wright D. Down syndrome screening in the first and/or second trimester: model predicted performance using meta-analysis parameters. Semin Perinatol 2005;29:252–7.
[21] Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. J Med Screen 1997;4:181–246.
[22] Moertel CA, Stupca PJ, Dewald GW. Pseudomosaicism, true mosaicism, and maternal cell contamination in amniotic fluid processed with in situ culture and robotic harvesting. Prenat Diagn 1992;12:671–83.
[23] Leung TY, Spencer K, Leung TN, Fung TY, Lau TK. Higher median levels of free beta-hCG and PAPP-A in the first trimester of pregnancy in a Chinese ethnic group. Implication for first trimester combined screening for Down's syndrome in the Chinese population. Fetal Diagn Ther 2006;21:140–3.
[24] Spencer K, Heath V, El-Sheikhah A, Ong CY, Nicolaides KH. Ethnicity and the need for correction of biochemical and ultrasound markers of chromosomal anomalies in the first trimester: a study of Oriental, Asian and Afro-Caribbean populations. Prenat Diagn 2005;25:365–9.
[25] Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn 2011;31:7–15.
[26] Pajkrt E, de GIM, Mol BW, van LJM, Bleker OP, Bilardo CM. Weekly nuchal translucency measurements in normal fetuses. Obstet Gynecol 1998;91:208–11.
[27] Lo YMD, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485–7.
[28] Jiang F, Ren J, Chen F, Zhou Y, Xie J, Dan S, et al. Noninvasive fetal trisomy (NIFTY) test: an advanced noninvasive prenatal diagnosis methodology for fetal autosomal and sex chromosomal aneuploidies. BMC Med Genomics 2012;5:57.
[29] Lo YM, Tsui NB, Chiu RW, Lau TK, Leung TN, Heung MM, et al. Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. Nat Med 2007;13:218–23.
[30] Bianchi DW, Platt LD, Goldberg JD. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstet Gynecol 2012;119:890–901.
[31] Ehrich M, Deciu C, Zwiefelhofer T, Tynan JA, Cagasan L, Tim R, et al. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. Am J Obstet Gynecol 2011 Mar;204:205.e1–11.
[32] Chiu RW, Akolekar R, Zheng YW, Leung TY, Sun H, Chan KC, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. BMJ 2011;342:c7401.
[33] Mersy E, Smits LJ, van Winden LA, de Die-Smulders CE, South-East Netherlands NIPT Consortium, Paulussen AD, et al. Noninvasive detection of fetal trisomy 21: systematic review and report of quality and outcomes of diagnostic accuracy studies performed between 1997 and 2012. Hum Reprod Update 2013;19:318–29.