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Review Article

From Down syndrome screening to noninvasive prenatal testing: 20 years' experience in Taiwan

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Abstract

Down syndrome is the most common autosomal chromosome aneuploidy. The prenatal Down syndrome screening protocol has been known in Taiwan for the past 20 years. The maternal serum double markers required for the screening test was first implemented into the general prenatal check-up back in 1994, where it had around a 60% detection rate at a 5% false positive rate. The first trimester combined test was started in 2005, and the maternal serum quadruple test was introduced in 2008 to replace the previous double test. The overall detection rate for the current screening strategies (first trimester combined or second trimester quadruple test) in Taiwan ranges between 80% and 85% at a fixed 5% false positive rate. Noninvasive prenatal testing (NIPT) is the latest powerful fetal aneuploidy detection method and has become commercially available in Taiwan starting from 2013. The sensitivity and specificity for NIPT are very high (both over 99%) according to large worldwide studies. Our preliminary data for NIPT from 11 medical centers in Taiwan have also shown a 100% detection rate for Down syndrome and Edwards syndrome, respectively. Invasive chromosome studies such as amniocentesis or chorionic villus sampling cannot be replaced by NIPT, and all prenatal screening and NIPT results require confirmation using invasive testing. This review discusses the Down syndrome screening method assessments and the progress of NIPT in Taiwan.

Keywords: Down syndrome; first and second trimester screening; noninvasive prenatal testing

Introduction

Down syndrome (DS) is the most common structural chromosome abnormality, with an occurrence rate of around 1 in 800 [1,2]. The initial DS screening tool was used for advanced maternal age patients (i.e., those over 35 years old) in the early 1980s; however, only 30% of DS patients could be detected by amniocentesis [3]. Second trimester maternal serum screening was introduced in the early 1990s using free human beta chorionic gonadotropin (free β-hCG) and alpha-fetoprotein (AFP) that significantly improved fetal aneuploidy screening performance [4–6]. In Taiwan, we first implemented the second trimester double test (free β-hCG and AFP) in 1994 as part of the routine prenatal check-up for every pregnant woman; this step was accompanied by a dramatic decrease of DS live birth rates from 0.63 to 0.16 per 1000 live births [7].

DS screening first started to shift to the first trimester combined test worldwide from the late 1990s and early 2000s; the combined test included the measurement of nuchal translucency thickness (NT), human chorionic gonadotropin (hCG)

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and pregnancy-associated plasma protein-A (PAPP-A) [8,9] levels. The overall detection rate was higher than the second trimester double test (85% vs. 60%) while maintaining the same 5% false positive rate [4]. In Taiwan, we implemented the first trimester combined test back in 2005 after some pioneers obtained NT scanning licenses from the Fetal Medicine Foundation (FMF) in London [8].

Recently in Taiwan, the maternal serum quadruple screening test was started using two markers plus unconjugated estriol (uE3) and inhibin A from 2008 to replace the insufficient detection power of the traditional double test [10]. The overall detection rate is 81% at a 5% false positive rate [11,12]. The quadruple test for DS screening is mainly applicable for general urban hospital populations, especially for those individuals lacking qualified NT scanning doctors. Currently, the first trimester combined test and second trimester quadruple test are the main tools for routine prenatal care in Taiwan [10].

NIPT has been a diagnostic tool for fetal chromosome aneuploidies since Lo et al first found free fetal cell DNA in the maternal plasma [13]. Chiu et al led this research and launched a clinical service starting in 2011 [14–16]. The overall DS detection specificity and sensitivity are both over 99%, as compared to 99.99% by invasive amniocentesis [17]. In Taiwan, NIPT is now commercially available, showing 100% DS detection compatible with other international studies [18]. In this review, we summarize DS screening trends and demonstrate the pilot data of NIPT screening in Taiwan.

Second trimester double test (1994)

The second trimester double test was the first DS screening test; it was introduced to Taiwan approximately 20 years ago [6]. It is generally offered between the 15th and 20th week of gestation. It traditionally consists of some combination of maternal serum marker analysis and maternal age. In the 1980s, researchers found that lower maternal serum AFP levels in the second trimester were also associated with an increased risk of DS [19,20].

Starting in 1994, the double test using free β-hCG and AFP was introduced into routine prenatal examinations in Taiwan [6]. The three largest studies carried out in Taiwan showed that the double test detection rate ranged from 57% to 63%, with a false positive rate of between 5.3% and 6.5% (Table 1) [4–6,8,10–12,14,18,27,37–43]; these results are compatible to those from Caucasian population studies [4–7]. Our unpublished data also revealed that the double test detection rate among younger women was found to be only 45% with a false positive rate of 3.0%; these results were comparable to the 46% detection rate from the study by Wald et al [4,8]. When correlating the results of three major studies in Taiwan, the average detection rate among women of young maternal age (<34 years old) is 49% [8].

Routine screening always focuses on young mothers. In Taiwan, over 90% of women with advanced age choose genetic diagnostic testing [21]. Both the low cost of genetic diagnosis in Taiwan and poor support systems for intellectually disabled children have resulted in Taiwanese women preferring amniocentesis rather than risk a DS baby, despite the fetal loss rate for amniocentesis of nearly 1 in 300 [8].

Wald et al suggested that the maternal serum double test is no longer justified as a routine screening tool for DS, on the basis of relatively low detection rates and poor cost effectiveness [22]. Since 2008 in Taiwan, the quadruple test with higher detection ability has replaced most double testing after implementation into routine prenatal care [10].

First trimester combined test (2005)

First trimester screening is normally conducted between the 11th and 14th week of gestation. NT is a powerful sonographic marker for DS, and free β-hCG and PAPP-A are the discriminatory serum factors [23,24]. These three markers are used for calculating the likelihood ratio, and thus determine individual risk of fetal DS [25]. The DS detection rate if performing NT alone ranged from 64% to 70% with a 5% false positive rate, depending on the study [11]. A combination of NT measurement with the above two serum biochemical markers (free β-hCG and PAPP-A) in the first trimester comprises the "combined test" [25]. The detection rate for this test is between 82% and 87% with a false positive rate of 5%; this is even better than the second trimester quadruple test [11]. Tsai et al published the first Taiwanese pilot data on the first trimester combined test which showed nine out of 10 fetal aneuploidies (including 2 DS) were diagnosed with a 4.7% false positive rate [26]. Chou et al noted that the detection rate for DS using the first trimester combined test was 87.5% (14 out of 16) with a false positive rate of 5.5% [27] (Table 1). Cheng et al reported that the homocysteine level would affect multiple of the median (MoM) of PAPP-A and adjustment of DS risk should be considered [28]. In addition, Yang et al also disclosed another soft marker (nasal bone length) during second trimester using three-dimensional ultrasound that could be applied for DS screening [29].

The first trimester combined test has the advantage of obtaining results in the late first trimester. When indicated, this allows karyotyping by chorionic villus sampling and early surgical termination of pregnancy. In 2007, the American College of Obstetrics and Gynecologists (ACOG) clinical

Table 1
Summary of the detection rate for Down syndrome screening and noninvasive prenatal testing in Taiwan versus literature findings (with around 5% false positive rate).

	Detection rate (Taiwan), % (*n*)	References	Detection rate (literature), % (*n*)	References
Second trimester double test	56 (34/61)	[5,6,8]	61	[4]
First trimester combined test	87.5 (14/16)	[27]	82–87	[11,12]
Second trimester quadruple test	81.8 (9/11)	[10]	81	[11,12]
Noninvasive prenatal testing	100 (11/11)	[18]	99.3 (590/594)	[14,37–43]

guidelines concluded that the first trimester combined test was an effective screening test for DS for the general population (level A evidence) [12]. The first trimester combined test was introduced at major medical centers in Taiwan from the beginning of 2005 following the FMF guidelines [8]. In 2013, there are over 50 FMF certified sonographers in Taiwan. The Department of Health in Taiwan also suggested that the first trimester combined test should officially be incorporated into routine care. One study showed that the first trimester combined test was the most cost-effective screening tool [30].

Currently in Taiwan, the first trimester combined test acts as the first-line DS screening in hospitals with certified doctors or sonographers. The second trimester quadruple test acts as the second-line screening tool when pregnant women are either late for prenatal check-ups or in local clinics without licensed doctors.

Second trimester quadruple test (2008)

One medical center in Taiwan studied uE3, in addition to hCG and AFP as the third marker (triple test); they found an overall DS detection rate of 78.6% [31]. Incorporating inhibin A into the maternal serum DS screening in the second trimester, along with AFP, hCG and uE3, was termed the quadruple test, and was first used in 1996 [32]. The quadruple test detection rate is approximately 83%, comparable with the first trimester combined test [11].

According to the ACOG published guidelines, triple or quadruple testing should be offered to pregnant women when certified doctors are not available to perform a first trimester combined test [12]. Starting in January 2008, the quadruple test began to be provided to the general population in Taiwan [10]. The standard reference range for inhibin A has never been determined among Asian women, therefore our pilot study to determine normal values was performed to establish a database [10]. Prior to performing maternal serum screening, informed consent should be offered to every pregnant woman. Comprehensive genetic counseling could help patients in their decision making after obtaining the results of DS screening [33].

Our data showed that 977 out of 21,481 women studied were in the high-risk group (over 1 in 270). Most of these women (86.2%) decided to have invasive procedures for genetic diagnosis. Nine cases of DS and 19 cases of other chromosomal anomalies were detected prenatally. Two children with DS were diagnosed post-delivery even though there was a low estimated risk, which was determined following the quadruple test. The detection rate was 81.8% (9 out of 11 cases), with a 4.4% false positive rate. The respective median multiple values for AFP, hCG, uE3 and inhibin A were 0.87, 2.34, 0.77 and 2.16 in the affected cases [10]. Therefore, Taiwanese data on second trimester quadruple testing showed compatible performances to other international studies (Table 1).

Noninvasive prenatal testing (2013)

Just recently, NIPT analysis of cell-free DNA (cfDNA) in maternal blood has been shown to be highly accurate in the detection of fetal chromosomal aneuploidies, such as autosomal or sex chromosome-related disorders [34]. The fetal DNA fraction is around 10–20% maternal plasma cfDNA and can be detected from the 10th week of gestational age onward [35]. There are several NIPT methods which include shotgun sequencing, massively parallel sequencing (MPS), target MPS, single nucleotide polymorphism-based approaches, methylated DNA-based approaches and digital PCR [34]. Currently, the most common method is shotgun MPS [15,36], which is based on the identification and counting of DNA fragment numbers in maternal plasma samples. With next generation sequencing, both fetal and maternal DNA fragments could be simultaneously sequenced for millions of times. The difference of DNA count numbers in chromosome 21 will show up in cases of DS, as compared to normal fetuses [15]. The calculation formula varies according to the different study groups. In general, the Z scores for each chromosome pair was defined as increased dosage when $Z > 3$, and decreased dosage when $Z < -3$ [15–17]. From the eight largest studies in the literature, the overall DS detection rate was 99.3% (590/594) with a 0.16% false positive rate [14,18,37–43] (Table 2).

Our pilot study was the first to demonstrate a prospective cohort study in the Chinese population using NIPT for detecting all fetal chromosomal aneuploidies [18]. It is also the first study to compare NIPT results from extremely high-/low-risk DS screening cases. There were 11 trisomy 21 cases, eight trisomy 18 cases, three trisomy 13 cases, one trisomy 16 case, three 45 XO cases and one 47 XYY case detected prenatally (Table 3). There were no false positive cases in this study. The detection rates for DS, trisomy 18, trisomy 13 and other autosomal aneuploidies were 100%, compatible to other international studies [14] (Tables 2 and 3). The false positive rate was zero, indicative that NIPT is a powerful screening tool. According to the latest ACOG guidelines, suggested NIPT indications are advanced maternal age, abnormal ultrasound findings and a positive result from either the first or second trimester screening [44]. The ACOG did not suggest using NIPT as the first-line routine prenatal evaluation, as NIPT is still more expensive as compared to other screening tools; it also had poor medical cost effectiveness. Invasive chromosome studies, such as amniocentesis, maintain a final confirmatory role.

Pregnant women, who are often afraid of invasive amniocentesis, have already had early rupture of membranes, multiple

Table 2
The detection of Down syndrome results in eight noninvasive prenatal testing studies, as compared with Taiwan data (modified from Benn et al [34]).

Trial [reference]	Detection rate, % (*n*)	False positive rate, % (*n*)
Chiu et al [14]	100 (86/86)	2.1 (3/146)
Ehrich et al [37]	100 (39/39)	0.24 (1/410)
Palomaki et al [38,43]	98.6 (209/212)	0.20 (3/1471)
Bianchi et al [39]	98.9 (89/90)	0.00 (0/410)
Sparks et al [41]	100 (36/36)	0.81 (1/123)
Ashoor et al [40]	100 (50/50)	0.00 (0/297)
Norton et al [42]	100 (81/81)	0.03 (1/2888)
Shaw et al [18]	100 (11/11)	0.00 (0/189)

Table 3
Summary of aneuploidies detected by noninvasive prenatal testing in Taiwan (modified from Shaw et al [18]).

Aneuploidies	Sensitivity, % (*n*)	Specificity, % (*n*)	False positive, %	False negative, %
Trisomy 21	100 (11/11)	100 (189/189)	0	0
Trisomy 18	100 (8/8)	100 (192/192)	0	0
Trisomy 13	100 (3/3)	100 (197/197)	0	0
Trisomy 16	100 (1/1)	100 (199/199)	0	0
45, X	75 (3/4)	100 (196/196)	0	25
47, XYY	100 (1/1)	100 (199/199)	0	0
Overall	96 (27/28)	100 (172/172)	0	4

pregnancies and good economic status could be clinical practical indications for NIPT [44]. NIPT may be used to detect one co-twin anomaly in twin pregnancies such as Turner syndrome or Edwards syndrome [18]. More data are necessary to confidentially apply such clinical testing in twin pregnancies. In the near future, NIPT might be helpful as a noninvasive procedure to assist with most parts of karyotyping.

NIPT currently remains limited in its ability to diagnose mosaicism, microdeletion, microduplication, translocations and triploidy [34]. Although one study claimed that NIPT could detect mosaicism [39], we still need to explain the application for all prospective clinicians who choose to perform NIPT. In addition, NIPT cannot detect balanced translocation as there is no DNA change in the entire fetal genome. Hence, chromosome karyotyping remains the gold standard for these patients. For other undetectable trisomies or triploidies, obstetricians are not worried because the previously mentioned diseases are too severe to survive in the fetus until birth; they are easy to find during prenatal ultrasound examinations.

In summary, next generation sequencing for NIPT could be efficiently applied in the general population for the detection of both autosomal and sex chromosome-related aneuploidies with very high sensitivity and specificity.

Conclusion

There has been 20 years of experience in Taiwan related to prenatal screening, ranging from DS screening to noninvasive prenatal testing. All clinical physicians have worked hard to improve prenatal care, as shown by the dramatic decreases in the DS live birth rate. We look forward to increased availability of high quality prenatal screening and diagnosis systems in Taiwan.

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