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Original Article

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

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Abstract

Background: A combination of maternal age and ultrasound assessment of the nuchal translucency (NT) has been used in the first trimester to screen for chromosomal abnormality. In the United Kingdom, the addition of NT screening was shown to be beneficial.

Aims: To report the sensitivity of combined first trimester biochemistry and ultrasound screening for Down syndrome in an Australian private practice specialising in obstetric ultrasound.

Methods: A prospective study in a private obstetric ultrasound practice. Over 22 months, 2121 patients were screened and data was analysed for sensitivity (detection) and false positive rates for all chromosome abnormalities.

Results: There were 17 chromosomal abnormalities, five of which were Down syndrome. Using maternal age alone or age and biochemistry, four of the Down syndrome cases were detected for a 29 and 19% false positive rate, respectively. Using age and NT or age, NT and biochemistry, all the Down syndrome cases were detected, for a false positive rate of 5.7 and 7.2%, respectively. The difference in detection rates for Down syndrome or other chromosomal abnormalities, using the four screening methods, did not reach statistical significance. However, the false positive rates in screening methods without ultrasound to assess the NT was significantly higher ($P < 0.01$).

Conclusions: A combination of maternal age, NT and maternal serum biochemistry gives a high detection rate for both trisomy 21 and other chromosomal abnormalities. Down syndrome screening using either maternal age alone or age in combination with first trimester biochemistry conferred screen positive rates significantly higher than when combined with NT.

Key words: Down syndrome, free beta human chorionic gonadotrophin, nuchal translucency, pregnancy associated plasma protein-A, screening.

Introduction

Screening for Down syndrome and other chromosomal abnormalities aims to provide women with the most accurate available risk assessment for them at that age in that particular pregnancy. The ideal screening test will achieve identification of the smallest possible high-risk group of pregnant women, with the largest proportion of affected pregnancies, at the earliest stage in pregnancy. Invasive testing is then offered and decisions can be influenced by the risk assessment.

Assessing risk based on maternal age enabled early invasive testing. Second trimester screening achieved better detection rates for Down syndrome and lower false positive rates than using maternal age alone.[1] However, diagnosis was only possible in the second trimester, when surgical termination of pregnancy is not routinely offered. First trimester screening became available with the discovery of the nuchal translucency (NT), a fluid-filled area posterior to the neck which tends to be larger in affected pregnancies and is best assessed between 11 and 14 weeks' gestation.[2]

The attractiveness of first trimester screening has been enhanced by the procedure related risk of transabdominal chorion villous sampling being shown to be similar to amniocentesis.[3] For the above reasons, women have shown a preference for first trimester screening.[4]

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A large multicentre study, from the Fetal Medicine Foundation at Kings College Hospital, achieved an 82% detection rate of pregnancies affected by trisomy 21 (Down syndrome) for a false positive rate of 8%.[2] They also detected 78% of other chromosomal abnormalities.

Of the biochemical markers that have been investigated, free beta-human chorionic gonadotrophin (fβ-hCG) and pregnancy associated placental protein-A (PAPP-A) have been shown to be of value in the first trimester.[5] A number of papers suggested the possibility of increased detection rates using a combination of maternal age, NT and biochemistry.[6,7] In 1999, a retrospective series of 210 cases of trisomy 21 showed a detection rate of 89% for a 5% false positive rate using a combination of age, NT and biochemistry assessing the levels of fβ-hCG and PAPP-A.[8]

There have been prospective international studies using age, NT and serology, showing detection rates approximately 90% for a 5% false positive rate.[4,9,10] The aim of the study was to establish the detection and false positive rates for an Australian population.

Methods

All women with a singleton pregnancy referred to Sydney Ultrasound for Women (SUFW) between the first of July 2000 and 3 May 2002 for first trimester screening for trisomy 21 were offered a combination of ultrasound for NT, maternal age and maternal serum fβ-hCG and PAPP-A levels. Patients were seen between 11 and 14 weeks' gestation, corresponding to a crown-rump length (CRL) on ultrasound of between 45 and 84 mm. Only in cases where the biochemistry sample was taken before the NT assessment were their data included. This avoided cases with a large NT measurement deciding on invasive prenatal testing without having the biochemistry or those with low risk on just age and NT, declining the addition of biochemistry. All women were made aware of the ongoing screening program audit and given a feedback form. The Human Research Ethics Committee of Sydney IVF approved the project, given as the clinical management was not altered by the study and it met all the requirements of the National Health and Medical Research Council definitions of quality assurance.

Maternal serum fβ-hCG and PAPP-A levels were measured using the Kryptor analyser (Brahms Diagnostica, Berlin, Germany). The laboratory, Sydney Genetics, used local data to create the curves from which to assess the multiples of the median (MOM) values for both analytes. Five hundred serum samples of pregnancies between 11 and 14 weeks' gestation were used, all of which had ultrasound dating. The MOM value for each analyte is corrected for maternal weight using another equation, as extremes of maternal weight have a significant effect.

Ultrasound examinations (ATL 5000; Philips, Seattle, USA) were performed to assess the fetal NT, CRL and to diagnose any major defects. The NT was measured in the sagittal plane, with the fetus taking in excess of two-thirds of the screen. The calipers were placed on the lines adjacent to the translucent area, using the technique outlined by the Fetal Medicine Foundation.[2] Sydney Ultrasound for Women is accredited by the Fetal Medicine Foundation and uses their risk analysis software, giving a gestational age specific risk, not a risk for an affected pregnancy at term. All patients were counselled to explain their risk assessment, and a risk of 1 in 300 or greater was classified as high risk. Patients were able to decide whether to have invasive testing, irrespective of their risk estimate.

The data from the pregnancies was obtained from referring doctors or the patients themselves via letter, phone, or a completed feedback form given at the time of the consultation.

The detection rate of trisomy 21 and other chromosomal abnormalities as well as the false positive rate was calculated using maternal age, age and biochemistry, age and NT or using all three parameters combined. The detection and false positive rates were compared with the χ^2 test to establish statistical significance, using $P < 0.05$.

Results

Screening for trisomy 21 was carried out on 2121 singleton pregnancies. Fetal NT and biochemistry was successfully measured in all cases. Pregnancy outcome, including karyotype or birth of a phenotypically normal baby was known in 2053 cases. Sixty-eight cases were excluded from analysis as no karyotype was available. These included stillbirths of unknown cause ($n = 2$), premature delivery ($n = 1$), termination of pregnancy for limb shortening ($n = 1$), spontaneous miscarriages ($n = 20$) and 44 lost to follow up. The age range of those lost to follow up was between 27 and 40 years, with nine being 35 years of age or over. None of the cases lost to follow up or those that miscarried were in the high-risk group.

The median age of the 2053 cases, at the time of testing, was 32 years (range: 15–44 years) and 602 (29%) were 35 years of age or older. The median CRL was 61 mm, with a range of 45–84 mm, correlating with a gestational age from 11 to 14 weeks. The median NT MOM was 1.01 and ranged from 0.08 to 5.51 (median: 1.4 mm, range: 0.1–10 mm). The median fβ-hCG MOM was 0.98 (range: 0.026–19.43) and PAPP-A MOM 0.88 (range 0.04–4.94).

Chromosomal abnormalities were identified in 17 pregnancies, including five with trisomy 21. Table 1 contains the maternal age, NT, fβ-hCG and PAPP-A MOM for each chromosome abnormality. All five trisomy 21 cases were detected using either age and NT or the combination of age, NT and biochemistry. This compares with using age and biochemistry or age alone which detected four of the five trisomy 21 cases ($P = 0.8$).

Twelve other chromosome abnormalities were identified. Seven of these were in the high-risk group using age and NT, eight using age, NT and biochemistry or age alone, and nine using age and biochemistry. These differences in detection rates were not statistically significant.

The false positive rate for the combination of age, NT and biochemistry was 7.2%. It was significantly higher for maternal age of 35 years or over (29%, $P < 0.01$) and for age

Table 1 Maternal age, NT and biochemistry for the chromosomal abnormalities

Age (years)	NT (MOM)	fβ-hCG (MOM)	PAPP-A (MOM)	Age and NT risk (1 in x)	Age and biochemistry risk (1 in x)	Age, NT and biochemistry risk (1 in x)	Chromosomal abnormality
32	5.51	0.66	0.36	9	469	10	Trisomy 21
36	2.72	1.91	0.54	8	60	3	Trisomy 21
37	4.15	2.57	0.66	4	42	3	Trisomy 21
38	4.75	0.71	0.47	3	225	5	Trisomy 21
39	1.71	0.75	0.46	73	131	124	Trisomy 21
33	3.12	0.48	0.18	8	69	5	Trisomy 13
37	4.23	0.24	0.29	3	290	6	Trisomy 18
42	7.99	0.72	0.3	2	17	2	Trisomy 18
33	3.69	0.57	0.31	7	284	6	Trisomy 22
36	0.89	4	0.18	1386	4	27	Trisomy 22
36	0.73	1.64	0.26	1519	12	96	Trisomy 22
27	2.40	7.48	2.21	83	82	11	Triploidy
34	0.73	0.11	0.08	2491	11	88	Triploidy
35	0.96	2.66	1.08	1738	155	1284	45X
40	1.60	0.92	1.18	73	373	513	47XXX
40	1.48	0.59	0.98	112	366	512	47XXY
37	1.09	0.57	0.8	1158	1062	1487	47XY + Marker

fβ-hCG, free beta human chorionic gonadotrophin; MOM, multiples of the median; NT, nuchal transluscency; PAPP-A, pregnancy associated placental protein-A.

Table 2 Detection of trisomy 21

Screening method	Number of trisomy 21 detected	Number in high-risk group	OAPPR	*P*-value compared with age, NT and biochemistry
Age	4/5	602	1 in 150	$P < 0.01$
Age and NT	5/5	117	1 in 23	$P = 0.7$
Age and biochemistry	4/5	394	1 in 99	$P = 0.06$
Age, NT and biochemistry	5/5	148	1 in 30	–

NT, nuchal translucency; OAPPR, odds of an affected pregnancy after a positive result.

and biochemistry (19%, $P < 0.01$), but similar for age and NT (5.7%, $P = 0.07$). The large difference in the false positive rates and small difference in detection rates affects the odds of an affected pregnancy after a positive result for trisomy 21 (Table 2).

The addition of biochemistry to the assessment by age and NT changes the risk and hence altered the composition of the high-risk group. The addition of biochemistry reduced the risk for some, enabling 55 patients to move from the high-risk group to the low-risk group. Other patients had their risk increased by biochemistry, moving 86 patients from the low-risk group to the high-risk group. In all, 141 patients changed risk groups, with 31 more ending up in the high-risk group increasing the false positive rate with the addition of biochemistry.

Using age, NT and biochemistry, 148 patients were considered to be at increased risk and 134 had invasive karyotyping. Of the 14 not having karyotyping, seven were under and seven over 35 years of age. In the low-risk group, 142 patients still had invasive karyotyping. Of these, 120 of which were over 35 years of age and of the remaining 22, only five had an increase in the age-related risk by the addition of the NT and biochemistry.

Discussion

The detection rate for Down syndrome achieved in this study is comparable with both the Bindra *et al.* and the von Kaisenberg *et al.* studies.[9,10] All five cases of trisomy 21 were detected using either age and NT or age, NT and biochemistry. The small case numbers did not allow any difference in detection rates between the two assessment methods to be evident, unlike the UK study.

The patients studied were self-selected to be an older population, with 29% at or above 35 years of age. Using maternal age alone as a screening tool would result in a large false positive rate (29%), which was only slightly reduced with the addition of biochemistry (19%). These large false positive rates result in poor positive predictive values for the detection

of trisomy 21 using age alone or age and biochemistry (1 in 150 and 1 in 99, respectively). The false positive rate dropped dramatically with the addition of ultrasound to assess the NT. This suggests that screening using maternal age alone or age and biochemistry should be combined with ultrasound to reduce the false positive rate, whenever this is possible.

It is worth noting that the median PAPP-A level was 0.88 MOM, rather than 1 MOM. This would have increased the false positive rate slightly. When the program was introduced into Australia, the MOM were calculated using the curves supplied by the Fetal Medicine Foundation, based on their data from London. New curves were recalculated using local data and the results monitored. By the end of the study period, the consistently low PAPP-A MOM were realised and new median values were recalculated using a population in excess of 15 000 patient's samples. The median PAPP-A and fβ-hCG MOM's in our ongoing program are consistently close to 1 MOM. This has reduced the false positive rate using age and biochemistry from 19 to 14%. Although this is an improvement, it is still much higher than with the addition of ultrasound. If the new median curve were used to recalculate the present study's data, none of the Down syndrome pregnancies would have moved out of the high-risk group. Hence, the detection rate would be unchanged for a lower false positive rate, using either age and biochemistry or age, biochemistry and NT (false positive response 14 or 6.5%, respectively).

The addition of biochemistry to screening with age and NT will alter the composition of the high-risk group. There are a number of disadvantages of performing the biochemistry after the ultrasound assessment. A significant proportion of patients will be moved either into or out of the high-risk group, which will require explanation at a later date when all the information is available. Many find this change in risk assessment difficult to understand, particularly if a low risk estimate from age and NT becomes a high-risk result a few days later due to the biochemistry.

Frequently patients with a low risk from age and NT decide not to have the biochemistry. These patients do not get the advantage of the biochemistry and the UK data suggests that some trisomy 21 cases will not be detected.[9] Results could be withheld until the biochemistry is available. This delays giving any of the available information and necessitates another visit for the consultation, neither appealing to patients and their partners.

Using age, NT and biochemistry, 90% of the high-risk group decided to have invasive testing. There were still 142 in the low-risk group who underwent invasive testing and of these 85% were 35 years of age or over. The majority of these women had the maximum reduction in risk that the testing was capable of producing, which was still not sufficiently reassuring to avoid definitive testing. Twenty-two women in the low-risk group who were under 35 years of age, had invasive testing, none of whom miscarried. In five of these cases the screening increased their risk above their age-related risk, which could explain why they were more concerned and decided to obtain a definitive result. The other 17 had no indication for invasive testing other than their desire for a definitive rather than a screening result. This shows that, while an expected detection rate of approximately 90% is reassuring, it is still not good enough for some couples.

The discriminatory ability of PAPP-A decreases with advancing gestation.[9] If the biochemistry is arranged after the ultrasound, then it will be at a later gestation, giving less discrimination between normal and trisomy 21 cases. The average PAPP-A level for trisomy 21 at 11 weeks (CRL 45 mm) is 0.4 MOM, by 12 weeks is 0.6 MOM, where as by 14 weeks (CRL 84 mm) it is 1 MOM, being the same as in normal pregnancies.[9] In the present study all the Down syndrome affected pregnancies had CRL measurements below 66 mm (equating to 12 weeks, 5 days gestation), so the biochemistry was collected at an early gestational age and the trisomy 21 cases had PAPP-A levels ranging between 0.36 and 0.66 MOM.

In conclusion, biochemistry performed before the ultrasound enables all the data to be discussed at the same visit, reducing confusion and anxiety. If the biochemistry is performed after the ultrasound, then the discriminatory ability of the PAPP-A will be reduced and another visit for a consultation must be arranged.

The results from our Australian population show a very high detection rate for both trisomy 21 and other chromosomal abnormalities using a combination of either maternal age and NT or maternal age, NT and biochemistry. The odds of a positive result were slightly better with age and NT than for age, NT and biochemistry, which is due to the false positive rates, but was not statistically significant. The cost of the biochemistry is not able to be justified on the result of the present study. However, a larger audit may have revealed a difference in detection rates as seen in the larger international studies.

Maternal age alone or age and biochemistry have high false positive rates, so should be used in conjunction with ultrasound to confirm the gestational age and assess the NT, whenever this is possible. The next enhancement to first trimester screening is likely to be the fetal nasal bone, which appears to be absent more commonly in Down syndrome pregnancies.[11]

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