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SHORT COMMUNICATION

The influence of fetal sex in screening for trisomy 21 by fetal nuchal translucency, maternal serum free β-hCG and PAPP-A at 10–14 weeks of gestation

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In a study of 2923 normal pregnancies and 203 pregnancies affected by trisomy 21 we have shown a significant difference in the median MoM of the markers: fetal nuchal translucency, maternal serum free β-hCG and PAPP-A in the presence of a female fetus compared with a male fetus. For maternal serum free β-hCG levels are higher by 15% if the fetus is chromosomally normal and by 11% if the fetus has trisomy 21. For maternal serum PAPP-A the levels in chromosomally normal fetuses are 10% higher in the presence of a female fetus and 13% higher if the fetus has trisomy 21. In contrast, fetal nuchal translucency is 3–4% lower in both chromosomally normal and trisomy 21 female fetuses. The consequence of such changes when screening for trisomy 21 will be a reduction in the detection rate in female fetuses by a factor of 1–2%. Correction of risk algorithms for fetal sex, however, is probably not feasible, since ultrasound detection of fetal sex is only 70–90% accurate in the 10–14 week period. Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS: nuchal translucency; free β-hCG; PAPP-A; prenatal screening; Down syndrome; first trimester

INTRODUCTION

In the second trimester of pregnancy, screening for trisomy 21 using maternal serum markers such as AFP and free β-hCG or total hCG is well established and although fetal sex has been known for some time to influence maternal serum levels of total hCG (Brody and Carlstrom, 1965) and AFP (Sowers *et al*., 1983), the implications for trisomy 21 detection rates and false-positive rates have only recently been considered (Bazzett *et al*., 1998; Ghidini *et al*., 1998; Spong *et al*., 1999; Spencer, 2000). Essentially, in the presence of female fetuses, maternal serum total and free β-hCG are increased by some 7% in chromosomally normal fetuses but not significantly different in trisomy 21 fetuses. Similarly with AFP, in the presence of female fetuses levels are 3% lower in chromosomally normal fetuses yet not significantly different in trisomy 21 fetuses. These differences lead to a higher false-positive rate in the presence of a female fetus.

There is now interest in moving screening for chromosomal abnormalities to the first trimester of pregnancy (Grudzinskas and Ward, 1997). Combining maternal serum pregnancy-associated plasma protein – A (PAPP-A) and free β-hCG with fetal nuchal translucency thickness (NT) can identify about 90% of pregnancies affected by trisomies 21, 18 and 13, triploidy, and sex chromosome aneuploidies for a false positive rate of about 5% (Spencer *et al*., 1999, 2000a, b, c; Tul *et al*., 1999). Prospective screening delivered in a one-stop clinic for assessment of risk for fetal anomalies (OSCAR) has been in place since 1998 and the early data from this clinic confirm the estimated performance from retrospective studies (Spencer *et al*., in press).

The aim of this study is to examine the influence of fetal sex on markers of trisomy 21 in the first trimester and how this may impact on detection and false-positive rates.

MATERIALS AND METHODS

Fetal NT and maternal serum free β-hCG and PAPP-A in chromosomally normal and trisomy 21 pregnancies were examined in relation to fetal sex. For the chromosomally normal group, data were derived from Harold Wood Hospital, Essex. In this hospital women booked for maternity care were given the option of attending an OSCAR for chromosomal defects by a combination of fetal NT and maternal serum free β-hCG and PAPP-A at between 10 weeks 3 days and 13 weeks 6 days (Snijders *et al*., 1998; Spencer *et al*., 1999; Spencer *et al*., in press). All biochemical measurements were performed using the CIS Kryptor rapid random access immunoassay analyser (CIS-UK Ltd, High Wycombe, Bucks, UK). Marker levels were converted to MoMs (with maternal weight correction where appropriate) using procedures outlined previously

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(Spencer *et al.*, 1999) and patient-specific risks were calculated according to the multivariate likelihood approach (Reynolds and Penney, 1990) using the population parameters in our retrospective study (Spencer *et al.*, 1999). All clinical information related to each pregnancy at the time of the OSCAR visit were recorded in a fetal database. Pregnancy outcome was obtained from patient records, delivery room records and cytogenetic registers.

Data for the trisomy 21 group were obtained from 203 affected cases, which formed part of a previous study (Spencer *et al.*, 1999), and 20 cases identified prospectively in the OSCAR clinic at Harold Wood Hospital, Essex and at the Fetal Medicine Centre, London.

All statistical analyses were performed with Analyse-It (Smart Software, Leeds, UK) a statistical software add-on for Microsoft Excel 7. In order to simulate the impact of fetal gender on detection and false-positive rates in routine screening practice we used standard statistical modelling techniques (Royston and Thompson, 1992). We used the population parameters for both affected and unaffected groups outlined in our previous study (Spencer *et al.*, 1999). A series of 15 000 random MoM values were generated from within the distributions of the affected and unaffected pregnancies. These values were used to calculate the reference likelihood ratios for the three marker combination (fetal NT, maternal free β-hCG and PAPP-A). The likelihood ratios were then used together with the age-related risk for trisomy 21 in the first trimester (Snijders *et al.*, 1994) to calculate the expected detection rate of affected pregnancies, at a fixed false-positive rate, in a population with the maternal age distribution of pregnancies in England and Wales (Office for National Statistics, 1998). To assess the impact of fetal gender the 15 000 generated MoMs were adjusted to represent female values by the observed gender differences, and these adjusted MoMs were then used to calculate new likelihood ratios and detection rates as described above.

RESULTS

During the period 1 June, 1998 to 31 May, 1999 fetal sex information was available from 2923 women who delivered a single healthy infant at Harold Wood Hospital (Table 1) and in whom no pregnancy complications (pre-eclampsia, IUGR, pregnancy hypertension, low birth weight, fetal structural anomalies or fetal chromosomal anomalies) were observed.

The female to male median MoM ratios for free β-hCG, PAPP-A and NT by gestational week are shown in Table 2. When the $\log_{10}$ marker MoMs were compared between fetal sexes using *t*-tests assuming equal variance, in the presence of a female fetus maternal serum free β-hCG and PAPP-A were higher by 15% and 10%, respectively ($p<0.00001$), whereas fetal NT was lower by 3% ($p<0.01$) and the median MoM ratios were consistent across each gestational week (Table 2).

Table 1—Characteristics of the unaffected study population, median (range)

	Female fetus $n=1393$	Male fetus $n=1530$
Maternal weight (kg)	64.6 (40.6–130)	65.9 (41–135)
Maternal age (years)	29.0 (15.4–45.7)	29.2 (16.2–44.1)
Gestation (days)	84 (73–97)	85 (73–97)
Smokers/non smokers	175/1103	285/1226

Table 2—Ratio of median marker levels at various gestational weeks classified by the presence of a male or female fetus

Gestation (weeks)	10	11	12	13	All
Number of females	106	527	587	173	1393
Number of males	118	537	648	229	1530
Female : male free β-hCG MoM	1.260	1.162	1.171	1.028	1.152
Female : male PAPP-A MoM	1.018	1.082	1.121	1.076	1.102
Female : male NT MoM	0.953	0.971	0.974	0.979	0.972

In terms of screening for trisomy 21, the estimated risk was 1 in 300 or greater in 6.5% (91 of 393) of pregnancies with a female fetus and 6.9% (105 of 1530) with a male fetus (Chi-square 0.797, $p=0.78$). Thus, women carrying a male fetus were 1.05-times more likely to be in the 'at increased risk' group than those carrying a female fetus.

In the trisomy 21 group (Table 3) the proportion of males to females was 1.28, and this was similar to that observed in national registers (Mutton *et al.*, 1996). When the markers were analysed by fetal sex the median MoM NT in the presence of a female trisomy 21 fetus was 4% lower than with a male fetus (2.536 versus 2.636); when the $\log_{10}$ NT MoMs were compared between fetal sexes using *t*-tests, assuming equal variance, this difference was significant ($p=0.018$). For free β-hCG the median MoM in the presence of a female trisomy 21 fetus was 11% higher than with a male fetus (2.129 versus 1.921); when the $\log_{10}$ free β-hCG MoMs were compared between fetal sexes using *t*-tests, assuming equal variance, this difference did not quite reach statistical significance ($p=0.067$). For PAPP-A the median MoM in the presence of a female trisomy 21 fetus was 13% higher than with a male fetus (0.554 versus 0.489); when the

Table 3—Characteristics of the trisomy 21 study population, median (range)

	Female fetus $n=98$	Male fetus $n=125$
Maternal weight (kg)	65.0 (48–118)	62.0 (44–133)
Maternal age (years)	37.0 (19–45)	38.0 (22–45)
Gestation (days)	85.9 (73–97)	86.7 (75–97)
Smokers/non smokers	15/83	11/114

$\log_{10}$ PAPP-A MoMs were compared between fetal sexes using *t*-tests, assuming equal variance, this difference was not significant ($p = 0.153$).

The pattern of change in marker levels in cases of trisomy 21 are similar to those in the unaffected group. The likely effect of a lower NT and higher PAPP-A would be to reduce the chance of detection of a female fetus with trisomy 21. Although this would be partially balanced by the higher free β-hCG, the overall detection rate in the presence of a female fetus would be expected to be lower. In the population simulation study, at a 5% false-positive rate, the detection rate in females was lower than in males by 1.5% (87.5% versus 89.0%).

DISCUSSION

The findings of this study demonstrate that, in first-trimester pregnancies with female fetuses, compared with male fetuses, maternal serum free β-hCG levels are higher by about 15% if the fetus is chromosomally normal and by 11% if the fetus has trisomy 21. In the second trimester, free β-hCG in pregnancies with female fetuses is also increased both in amniotic fluid (Spencer *et al*., 1997) and maternal serum, but this increase was not observed in trisomy 21 pregnancies (Spencer, 2000).

This study has also demonstrated that in first-trimester pregnancies with female fetuses, compared with male fetuses, maternal serum PAPP-A levels are higher by about 10% if the fetus is chromosomally normal and by 13% if the fetus has trisomy 21. In contrast, fetal NT is about 3–4% lower in both chromosomally normal and trisomy 21 female fetuses.

The implication of the observed fetal gender-related changes in fetal NT and maternal serum free β-hCG and PAPP-A on first-trimester screening for trisomy 21 would be a 1–2% lower detection rate in the presence of a female fetus. This can potentially be corrected, because it is now possible to determine the fetal sex by ultrasound examination at 10–14 weeks of gestation (Efrat *et al*., 1999; Whitlow *et al*., 1999). However, at present the accuracy of fetal gender assignment by ultrasound is only 70–90%, depending on the week of gestation, and therefore, it would not be possible to consistently correct for fetal gender at the time of the OSCAR visit. At this present moment we would not recommend correction for fetal sex in either the first or second trimester.

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