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Screening for trisomy 21 in twin pregnancies in the first trimester: an update of the impact of chorionicity on maternal serum markers

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Objective To examine the distribution of first-trimester biochemical markers of aneuploidy in twin pregnancies, and to assess whether there are differences in the distributions between monochorionic and dichorionic twins.

Methods Maternal serum-free β-hCG and PAPP-A were measured between $11+0$ and $13+6$ weeks as part of a routine first-trimester screening program in conjunction with fetal nuchal translucency (NT) performed at two sites. Data from twin pregnancies were extracted from the fetal databases along with information on the chorionicty. The individual marker concentrations were expressed as weight corrected, ethnicity corrected, smoking corrected and IVF corrected MoM using data from singleton pregnancies as the reference. The overall medians were compared to those in singleton pregnancies and between monochorionic and dichorionic twins.

Results Data was available from 1914 sets of twins. Of these, 1214 had information with respect to chorionicity, with 1024 being dichorionic and 190 being monochorionic. The overall median weight corrected, ethnicity corrected, smoking corrected and IVF corrected MoM amongst twin pregnancies were 2.023 for free β-hCG (sd $\log_{10}$ MoM = 0.2611 and 2.121 for PAPP-A (sd $\log_{10}$ MoM = 0.2255)—both medians were significantly greater than the medians in singleton pregnancies (1.00 MoM). In the case of monochorionic and dichorionic twins the median weight corrected, ethnicity corrected, smoking corrected and IVF corrected, free β-hCG MoM's were not significantly different (1.983 v 2.041), however for PAPP-A the median weight corrected, ethnicity corrected, smoking corrected and IVF corrected MoM in monochorionic twins was significantly lower than in dichorionic twins (1.756 v 2.250) whilst the sd $\log_{10}$ MoM's were not significantly different (0.2185 v 0.2167).

Conclusion Screening in twin pregnancies requires adjustment of the calculated MoM to account for the presence of two fetuses. In general, for free β-hCG, this should be by dividing the observed corrected MoM by 2.023. For PAPP-A two different factors are required −2.192 in dichorionic twins and 1.788 in monochorionic twins. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: PAPP-A; free β-hCG; prenatal screening; twins; Down syndrome

INTRODUCTION

First-trimester screening using a combination of fetal nuchal translucency (NT) thickness and maternal serum-free β-hCG and PAPP-A has been shown in retrospective (Spencer *et al*., 1999) and prospective studies (Nicolaides *et al*., 2005) to identify 90% of cases of trisomy 21 with a 5% false positive rate in singleton pregnancies. In twin pregnancies, both NT (Sebire *et al*., 1996) and maternal serum biochemistry can be combined (Spencer, 2000) to provide detection rates that would approach those achieved in singleton pregnancies. Whilst these early algorithms were based on relatively small numbers of cases of twins, retrospective use has shown them to be useful in identifying pregnancies discordant for trisomy 21 (Spencer and Nicolaides, 2000, 2003).

An initial study (Spencer, 2001) which looked at the influence of chorionicity on biochemical marker levels concluded that there was no statistically significant difference between marker levels in monochorionic and dichorionic twins—despite there being the suggestion of lower values of PAPP-A in the former case. Subsequent small studies have confirmed no difference in marker levels between mono and dichorionic twins (Gonce *et al*., 2005; Wojdemann *et al*., 2006).

The present study was undertaken to provide more secure information on the distribution of biochemical markers in twin pregnancies and to establish if the previously observed difference in median PAPP-A MoM in monochrorionic twins is significant in a larger series.

MATERIALS AND METHODS

All women booked for maternity care at the following UK hospitals were offered screening for trisomy 21 by a combination of fetal NT and maternal serum-free

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β-hCG and PAPP-A at 11^{+0} to 13^{+6} weeks' gestation: Harold Wood Hospital, Romford (between June 1998 and December 2005), King George Hospital, Goodmayes (between July 2001 and December 2005), Kent and Canterbury Hospital, Canterbury (between July 2002 and December 2005), William Harvey Hospital, Ashford (between July 2002 and December 2005), Queen Elizabeth The Queen Mother's Hospital, Margate (between July 2002 and December 2005), King's College Hospital, London (between January 1999 and February 2000) and those attending The Fetal Medicine Centre, London (between July 1999 and June 2005). Women received an information leaflet about the service and gave details about their demographic characteristics and medical history, which were entered into a computer database (PIA-Fetal Database, ViewPoint, Webling, Germany).

Maternal serum-free β-hCG and PAPP-A were measured using the Kryptor analyzer (Brahms AG, Berlin, Germany) as previously described (Spencer *et al*., 1999) and an ultrasound examination was carried out to measure fetal NT and crown–rump length (CRL) and to diagnose any major fetal abnormalities. All scans were carried out by sonographers who had obtained The Fetal Medicine Foundation Certificate of Competence in the 11^{+0} to 13^{+6} week scan (www.fetalmedicine.com). At the Harold Wood Hospital, King George Hospital and the Fetal Medicine Centre chorionicity was recorded in twin pregnancies by the identification of the lambda sign in dichorionic twins (Monteagudo *et al*., 1994; Sepulveda *et al*., 1996, 1997). Patient-specific risks were calculated by a multivariate approach using biochemical population parameters outlined in a previous study (Spencer *et al*., 1999), likelihood ratios based on delta NT outlined in a previous study (Spencer *et al*., 2003a) and the gestational age-related risk of trisomy 21 at the time of screening (Snijders *et al*., 1999). Data on pregnancy outcome were obtained from the cytogenetics laboratories, the National Chromosomal Anomaly Register, the patients themselves, their general practitioners or the maternity units in which they delivered.

The measured free β-hCG and PAPP-A were converted to a multiple of the expected normal median (MoM) for a pregnancy of the same gestational day using values established in a previous study (Ong *et al*., 2000) corrected for maternal weight (Spencer *et al*., 2000, 2003b), ethnicity (Spencer *et al*., 2005), smoking (Spencer *et al*., 2004) and assisted reproduction (Liao *et al*., 2001). Gestational age was calculated from the CRL of the larger twin.

The fetal database was searched for records of live-born twin pregnancies, which had complete outcome information, including gestational age at delivery (completed weeks), and pregnancy outcome.

Statistical analysis of corrected PAPP-A and free β-hCG MoM's in all cases of twins, and in cases classified as monochorionic or dichorionic were performed using *t*-test of unequal variance with the $\log_{10}$ MoMs and using nonparametric Mann-Whitney tests on the untransformed MoMs using Analyse-It (Leeds), a statistical add-in for Microsoft Excel.

RESULTS

Totally, data from 1914 sets of twins were available for analysis, and of these, 1024 were dichorionic, and 190 were monochorionic. This dataset includes those previously published (Spencer, 2000; Spencer and Nicolaides, 2003). Table 1 summarizes the characteristics of the group. The overall median corrected MoM in the twin dataset was 2.023 (95% confidence Interval (CI) 1.958–2.085) for free β-hCG and 2.121 (95% CI 2.080–2.191) for PAPP-A. The standard deviation of $\log_{10}$ free β-hCG was 0.2611 and for PAPP-A was 0.2255.

When we examined the distributions according to chorionicity the median MoM-free β-hCG was not significantly different between monochorionic (1.983; 95% CI 1.794–2.195) and dichorionic twins (2.041; 905% CI 1.932–2.130) when tested using Mann-Whitney ($p = 0.9278$). When the $\log_{10}$ MoMs were examined by *t*-test again there was no difference ($p = 0.7626$) and the sd's were 0.3060 and 0.2512 respectively. For PAPP-A, however, there was a highly significant difference in the distribution between monochorionic (1.756; 95% CI 1.651–1.868) and dichorionic twins (2.250; 95% CI 2.152–2.337) when tested using Mann-Whitney ($p < 0.0001$). When the $\log_{10}$ MoMs were examined by *t*-test again there was a significant difference ($p < 0.0001$) with the sd's being 0.2167 and 0.2185 respectively.

DISCUSSION

This study has confirmed for the first time that the distribution of PAPP-A in monochorionic twins is lower than that in dichorionic twins and builds upon our earlier suspicion of such a difference (Spencer, 2001). In our former study of 135 dichorionic and 45 monochorionic twins we found a lower median MoM PAPP-A in the monochorionic twins (1.66 v 1.89), which did not reach statistical significance. In another small study of 150 dichorionic twins and 31 monochorionic twins, Wojdemann *et al*., 2006 found no difference in marker levels between the two groups, but found an overall median MoM of 2.14 for PAPP-A (log sd 0.248) and 2.06 for free β-hCG (log sd 0.230). Niemimaa

Table 1—Characteristics of the twin population

Median maternal age (years)	32.9
Median gestational age (Weeks)	12.4
Mean crown-rump length (mm)	62.7
Twin 1	62.8
Twin 2	62.6
Afro-Caribbean (%)	3.76
Asian (%)	5.64
Oriental (%)	0.78
Caucasian (%)	84.48
Other (%)	1.10
Not stated (%)	4.23
Smoker (%)	10.29
IVF pregnancy (%)	14.00

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et al. (2002) in a study of 54 dichorionic and 13 monochorionic twins also found no significant difference in biochemical marker levels between the two groups with an overall median-free β-hCG MoM of 1.85 and 2.36 for PAPP-A. Gonce *et al*. (2005) in a study of 100 twin pregnancies comprizing 87 dichorionic and 11 monochorionic twins, found no significant difference in serum markers between the two groups. However, the overall median-free β-hCG MoM was lower than observed in other series (1.57, log sd 0.2614) whilst that for PAPP-A was similar to others (1.96, log sd 0.2465).

In summarizing the world series of data on twins (Spencer, 2005) in 825 cases, the median-free β-hCG was 2.035, and in 707 cases the median PAPP-A was 1.826. If the current data are added into this the median-free β-hCG in 2356 cases become 2.012, and in 2238 cases the median PAPP-A was 2.072.

Screening in twins by biochemistry alone is considered problematical by some (Cuckle, 1998), despite evidence that second-trimester screening can clearly distinguish around 50% of cases discordant for trisomy 21 (Spencer *et al*., 1994). In the first trimester, NT clearly has a much greater role to play in being able to provide an individual or fetal risk rather than biochemistry alone, which provides a pregnancy risk. Whilst NT can be used successfully to screen in twins with a similar detection rate and false positive rate to that in singleton pregnancies, the combination of both first-trimester NT and maternal serum biochemistry can improve the overall detection rate to around 80% (Spencer, 2000) using the pseudo-risk approach, and this has been borne out in prospective screening practice (Spencer and Nicolaides, 2003). Other alternative strategies involving pregnancy-specific risks have been proposed (Wald *et al*., 2003) which use estimated distributions of markers in twins rather than making correction for twins and the calculation of a singleton equivalent or pseudo risk. The problem with this approach is the sparse data available to determine the distribution of markers in twins concordant or discordant for trisomy 21. In a meta-analysis of the world published series Spencer (2005) was only able to show 6 concordant cases and 23 discordant cases.

The data from this present study will enable more accurate twin risks to be produced, and in pregnancies where chorionicity is known, then the use of a specific monochorionic twin correction factor along with the use of the average NT measured in the two fetuses or the average fetal risks (Vandecruys *et al*., 2005) will improve the accuracy of the individual patient-specific risk.

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