PRENATAL DIAGNOSIS
Prenat Diagn 2005; **25**: 47–50.
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/pd.1082

Down syndrome screening marker levels in women with a previous aneuploidy pregnancy

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Background In Down syndrome screening programmes, women with a previous affected pregnancy are assumed to have the same marker distribution as those without a family history. This assumption needs to be tested.

Methods Information on previous aneuploidy pregnancies was routinely sought on the test request forms in three centres, Leeds, Romford and the Fetal Medicine Centre, London. For each woman with a previous aneuploidy (case), five unaffected pregnancies to women without a history were selected as controls. The markers tested included maternal serum free β-human chorionic gonadotrophin (hCG), pregnancy-associated plasma protein A (PAPP-A), α-fetoprotein, unconjugated estriol and ultrasound nuchal translucency thickness.

Results There were 375 cases: 303 with previous Down syndrome, 63 with Edwards syndrome and 9 with Patau's syndrome. There was a statistically significant difference between cases and controls, in the distribution of free β-hCG and PAPP-A levels, adjusted for gestation. On average, free β-hCG was increased by 10% in a subsequent pregnancy after aneuploidy ($p < 0.005$, Wilcoxon rank sum test) and for PAPP-A the increase was 15% ($p < 0.0001$). No other marker was significantly different.

Conclusion Risk calculation algorithms need to be modified to take account of the increased marker levels. Until data from sufficient affected pregnancies are available for study, it would be prudent to assume that the same increase as in unaffected pregnancies applies. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: Down syndrome; screening; markers; previous aneuploidy

INTRODUCTION

The standard method of interpreting a Down syndrome screening test is to estimate the risk of an affected pregnancy from the maternal age-specific risk and the marker profile. If the woman has had a previous pregnancy with aneuploidy, the normal age-specific risk will not apply. In a small proportion of cases, there will be a parental structural chromosome rearrangement and the recurrence risk can be quite high, depending on the specific parental genotype. In the vast majority of cases, the risk is more modest and can be readily calculated by adding a fixed amount to the age-specific risk, expressed as a percentage. One estimate for women with a previous Down syndrome pregnancy is the addition of 0.77%, 0.54% and 0.42% for risks calculated in the first trimester, second trimester and at term respectively (Cuckle and Arbuzova, 2004). Similar corrections apply for Edwards and Patau's syndrome (Nicolaides *et al*., 1999). Once the correct age-specific risk has been obtained, the screening-related risk is usually calculated in the same way as any other pregnancy. However, this assumes that the marker distributions are the same in women with and without a previous aneuploidy pregnancy, and we now have evidence that this is not the case.

MATERIALS AND METHODS

Information on previous aneuploidy pregnancies is routinely sought on the Down syndrome screening test request forms used in Leeds, Romford and the Fetal Medicine Centre (FMC), London. In Leeds, those requesting either first or second trimester tests are asked about previous pregnancies with Down syndrome or Edwards syndrome. In Romford and FMC, the request is only made in the first trimester, but information about a previous Patau's syndrome is also sought.

In Leeds, women screened in both the first and second trimester are tested for maternal serum free β-human chorionic gonadotrophin (hCG), α-fetoprotein (AFP) and unconjugated estriol (uE_3) using time-resolved fluorescent assay (DELFIA®, Perkin-Elmer Life Sciences, Turku, Finland). In addition, all first-trimester screening tests included pregnancy-associated plasma protein A (PAPP-A) measurement (DELFIA®) and ultrasound nuchal translucency (NT) thickness. Among those screened in the second trimester, a proportion also had inhibin measured, but there were too few with a previous aneuploidy to be included in the study. In Romford and FMC, first-trimester screening tests are based on

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Received: 15 July 2004
Revised: 4 October 2004
Accepted: 25 October 2004

maternal serum free β-hCG and PAPP-A, measured by time-resolved amplified cryptate emission (Brahms AG, Berlin, Germany), together with NT.

All serum markers were expressed in multiples of the median for the appropriate gestation (MoMs) and corrected for maternal weight. The NT values from Leeds were also expressed in MoMs using either the general median equation published by Nicolaides *et al.* (1998), or, more recently, centre-specific medians (Logghe *et al.*, 2003), whereas those from Romford and FMC were expressed in deviations from the gestation-specific median (delta-values), as outlined by Spencer *et al.* (2003) using the general equation.

A total of 375 unaffected pregnancies to women with a previous aneuploidy were available for study. For each case, five pregnancies to women without previous aneuploidy were selected as controls. Matching criteria were same age, within 2 years, and same period of testing, within 4 weeks.

The non-parametric Wilcoxon rank sum test was used to make comparisons between the distribution of marker levels in cases and controls.

RESULTS

Table 1 shows the median marker level in cases and controls. There was a statistically significant increase in the level of both free β-hCG and PAPP-A among women who had a previous affected pregnancy compared to other women. Overall, the increase was 10% for free β-hCG and 15% for PAPP-A.

The PAPP-A result was significant in each centre separately and for each type of previous aneuploidy. The median PAPP-A level was higher in Edwards and Patau's than in Down syndrome, but there was no statistically significant heterogeneity ($p = 0.19$, Kruskal–Wallis Test).

The free β-hCG result was significant in Leeds, but not in Romford and FMC, and for Down and Edwards, but not for Patau's syndrome. As for PAPP-A, the median was higher in Edwards and Patau's than in Down syndrome, but there was no statistically significant heterogeneity ($p = 0.54$).

In Leeds, 146 of the cases had free β-hCG tests in the first trimester and 46 in the second trimester. The medians were 1.17 MoM ($p < 0.01$) and 1.10 MoM ($p = 0.82$), respectively. There was insufficient data to conclude about any possible interaction between the effect and trimester.

No material difference between cases and controls was found for any of the other markers. Moreover, when the Leeds AFP and uE_3 results were compared within trimester, no significant differences were found.

In addition to the median level, the spread of free β-hCG also differed between cases and controls. The standard deviation of $\log_{10}$ values, estimated from the 10 to 90th centile range, was 0.281 in cases and 0.254 in controls ($p < 0.01$, F-test for the variance ratio). In contrast, the spread of PAPP-A was similar in cases and controls, with standard deviations of 0.233 and 0.236 respectively.

DISCUSSION

We have shown that free β-hCG and PAPP-A levels are increased on average in unaffected pregnancies to women with a previous affected pregnancy.

With existing risk calculation algorithms the free β-hCG increase will lead to over-estimated risks, whilst the PAPP-A increase will reduce risks. A simple way of correcting this would be to divide the free β-hCG level by 1.10 and PAPP-A by 1.15, then calculate risks in the same way as usual, assuming that the proportional increase in levels applies equally to affected and unaffected pregnancies. Whilst there is as yet insufficient data to test this assumption, experience with other screening co-variables indicates that it is likely to be valid.

When both markers are measured, as in first-trimester screening, whilst the two effects will tend to cancel each other out, a corrected risk will be more accurate than one that is not corrected. For example, a 36-year-old woman with a previous affected pregnancy, 1.5 MoM free β-hCG and 1.0 MoM PAPP-A at 10 weeks, and 1.5 MoM NT at 11 weeks' gestation has a 1 in 260 Down syndrome risk at term, using the parameters in Cuckle and Arbuzova (2004). Correcting the serum levels increases the risk to 1 in 240. When only one marker is measured, as in second-trimester screening, the correction would be more important, although our study included too few second-trimester cases to be certain that the magnitude of the free β-hCG effect is the same in both trimesters.

One potential bias in our results is that cases are by definition parous, whereas controls could be nulliparous, and both hCG and PAPP-A levels vary with parity or gravidity (for a review, see Wald *et al.*, 1998; Spencer *et al.*, 2000). However, this is unlikely to be a large effect since, by matching on maternal age, we will have minimised the parity difference, and the association between the markers and parity is weak. Information on parity has been routinely sought on the test request form in Leeds since early 1996, so the increase in levels could be examined for cases tested in this period compared to their parous controls. The free β-hCG increase for 157 cases was 9% and the PAPP-A increase for 151 cases was 23%.

Maternal smoking also affects free β-hCG and PAPP-A levels (Wald *et al.*, 1998; Spencer, 1998, 1999; De Graaf *et al.*, 2000; Spencer *et al.*, 2004), and several studies have reported that smoking is less common in the mothers of infants with Down syndrome. However, a meta-analysis of all published studies to date has concluded that smoking does not confer a reduced risk (Rudnicka *et al.*, 2002), so this will not have biased our results.

Whilst maternal serum free β-hCG and PAPP-A are placental products, there is a maternal contribution to the level in an individual pregnancy. This is clear from the between-pregnancy correlations reported for total hCG (Holding and Cuckle, 1994; Dar *et al.*, 1996; Wax *et al.*, 2000), free β-hCG (Spencer, 1997, 2001; Spencer, 2002) and PAPP-A (Spencer, 2001; 2002). Maternal factors controlling the production or metabolism of these

Table 1—Median marker level in cases with previous aneuploidy and controls (MoMs except for NT in Romford/FMC, which is expressed in delta units)

	Previous pregnancy with syndrome				
	Down	Edwards	Patau's	Either	Control
Free β-hCG					
Leeds	1.15 (144)	1.15 (48)	—	1.15 (192)[a]	1.06 (960)
Romford/FMC	1.17 (159)	1.59 (15)[a]	1.30 (9)	1.22 (183)	1.07 (915)
Both	1.16 (303)[a]	1.32 (63)[a]	—	1.17 (375)[c]	1.06 (1875)
PAPP-A					
Leeds	1.23 (89)[c]	1.23 (29)[a]	—	1.23 (118)[d]	1.01 (591)
Romford/FMC	1.12 (159)	1.67 (15)	1.62 (9)[a]	1.18 (183)[b]	1.07 (915)
Both	1.14 (248)[c]	1.30 (44)[b]	—	1.21 (301)[e]	1.05 (1506)
NT					
Leeds	0.98 (91)	1.04 (30)	—	1.01 (91)	0.99 (598)
Romford/FMC	−0.10 (159)	−0.29 (15)	−0.28 (9)	−0.12 (183)	−0.14 (915)
AFP					
Leeds	1.06 (144)	0.99 (48)	—	1.06 (192)	1.04 (960)
uE_3					
Leeds	0.98 (144)	1.04 (48)	—	0.99 (192)	1.00 (960)

Statistical significance compared with controls (p): [a] < 0.05; [b] < 0.01; [c] < 0.005; [d] < 0.001; [e] < 0.0001.

analytes probably also impact on the increased levels in women with a previous aneuploidy.

Altered levels of several other analytes have been found in blood samples from women with previous aneuploidy pregnancies: neutrophil alkaline phosphatase (Vergnes *et al*., 1988), thyroid antibodies (Cuckle *et al*., 1998), Cu, Zn-superoxide dismutase (Arbuzova, 1998), follicle stimulating hormone (van Montfrans *et al*., 1999; Nasseri *et al*., 1999) and homocystine (James *et al*., 1999). It is possible that the same biological mechanism underlies both these and the changes we have observed. However, not all of these studies allowed fully for maternal age, as we have done, which may have confounded their results.

REFERENCES

Arbuzova S. 1998. Why it is necessary to study the role of the mitochondrial genome in trisomy 21 pathogenesis? *Downs Syndr Res Pract* **5**(3): 26–29.

Cuckle H, Arbuzova S 2004. Multianalyte maternal serum screening for chromosomal defects. In *Genetic Disorders and the Fetus: Diagnosis, Prevention and Treatment*, (5th edn), Milunsky Aubrey. (ed). Johns Hopkins University Press: Baltimore; 795–835.

Cuckle HS, Wald N, Stone R, Densem J, Haddow J, Knight G. 1998. Maternal serum thyroid antibodies in early pregnancy and fetal Down's syndrome. *Prenat Diagn* **8**: 439–445.

Dar H, Merksamer R, Berdichevsky D, David M. 1996. Maternal serum markers levels in consecutive pregnancies: a possible genetic predisposition to abnormal levels. *Am J Med Genet* **61**(2): 154–157.

De Graaf IM, Cuckle HS, Pajkrt E, Leschot NJ, Bleker OP, Van Lith JMM. 2000. Co-variables in first trimester maternal serum screening. *Prenat Diagn* **20**(3): 186–189.

Holding S, Cuckle HS. 1994. Maternal serum screening for Downs syndrome taking account of the result in a previous pregnancy. *Prenat Diagn* **14**: 321–322.

James SJ, Pogribna M, Pogribny IP, *et al*. 1999. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. *Am J Clin Nutr* **70**: 495–501.

Logghe H, Cuckle H, Sehmi I. 2003. Centre-specific ultrasound nuchal translucency medians needed for Down's syndrome screening. *Prenat Diagn* **23**: 389–392.

Nasseri A, Mukherjee T, Grifo JA, Noyes N, Krey L, Copperman AB. 1999. Elevated day 3 serum follicle stimulating hormone and/or estradiol may predict fetal aneuploidy. *Fertil Steril* **71**(4): 715–718.

Nicolaides KH, Sebire NJ, Snijders RJM. 1999. *The 11–14 week scan. The diagnosis of fetal abnormalities*, Parthenon: New York.

Nicolaides KH, Snijders RJ, Cuckle HS. 1998. Correct estimation of parameters for ultrasound nuchal translucency screening. *Prenat Diagn* **18**(5): 519–523.

Rudnicka AR, Wald NJ, Huttly W, Hackshaw AK. 2002. Influence of maternal smoking on the birth prevalence of Down syndrome and on second trimester screening performance. *Prenat Diagn* **22**(10): 893–897.

Spencer K. 1997. Between-pregnancy biological variability of maternal serum alpha-fetoprotein and free beta hCG: implications for Down syndrome screening in subsequent pregnancies. *Prenat Diagn* **17**(1): 39–45.

Spencer K. 1998. The influence of smoking on maternal serum AFP and free beta hCG levels and the impact on screening for Down's syndrome. *Prenat Diagn* **18**: 225–234.

Spencer K. 1999. The influence of smoking on maternal serum PAPP-A and free beta hCG levels in the first trimester of pregnancy. *Prenat Diagn* **19**(11): 1065–1066.

Spencer K. 2001. Between pregnancy biological variability of first trimester markers of Down syndrome: implications for screening in subsequent pregnancies. *Prenat Diagn* **21**: 445–447.

Spencer K. 2002. Between pregnancy biological variability of first trimester markers of Down syndrome and the implications for screening in subsequent pregnancies: an issue revisited. *Prenat Diagn* **22**: 874–876.

Spencer K, Bindra R, Cacho AM, Nicolaides KH. 2004. The impact of correcting for smoking status when screening for chromosomal anomalies using maternal serum biochemistry and fetal nuchal translucency thickness in the first trimester of pregnancy. *Prenat Diagn* **24**: 169–173.

Spencer K, Ong CYT, Liao AWJ, Nicolaides KH. 2000. The influence of parity and gravidity on first trimester markers of chromosomal abnormality. *Prenat Diagn* **20**: 792–794.

Spencer K, Bindra R, Nix ABJ, Heath V, Nicolaides KH. 2003. Delta NT or NT MoM: Which is the most appropriate method for calculating patient specific risks for trisomy 21 in the first trimester? *Ultrasound Obstet Gynecol* **22**: 142–148.

van Montfrans JM, Dorland M, Oosterhuis GJ, van Vugt JM, Rekers-Mombarg LT, Lambalk CB. 1999. Increased concentrations of

follicle-stimulating hormone in mothers of children with Down's syndrome. *Lancet* **353**: 1853–1854.

Vergnes H, Grozdea J, Brisson-Lougarre A, *et al*. 1988. An enzymatic marker in mothers of trisomy 21 children: neutrophil alkaline phosphatase. *Enzyme* **39**(3): 174–180.

Wald NJ, Kennard A, Hackshaw A, McGuire A. 1998. Antenatal screening for Down's syndrome. *Health Technol Assess* **2**(1): i–iv, 1–112.

Wax JR, Lopes AM, Benn PA, Lerer T, Steinfeld JD, Ingardia CJ. 2000. Unexplained elevated midtrimester maternal serum levels of alpha fetoprotein, human chorionic gonadotropin, or low unconjugated estriol: recurrence risk and association with adverse perinatal outcome. *J Matern Fetal Med* **9**(3): 161–164.