PRENATAL DIAGNOSIS
Prenat Diagn 2007; **27**: 849–853.
Published online 25 June 2007 in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/pd.1793

Dose dependency between cigarette consumption and reduced maternal serum PAPP-A levels at 11–13^{+6} weeks of gestation

K. O. Kagan[1], V. Frisova[1], K. H. Nicolaides[1] and K. Spencer[1,2]*

[1]*Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, London, UK*
[2]*Clinical Biochemistry Department, Harold Wood Hospital, Gubbins Lane, Romford, Essex, UK*

Objective To examine whether in smokers there is a significant dose dependency between the number of cigarettes per day and levels of free β-hCG and pregnancy-associated plasma protein A (PAPP-A) at 11–13^{+6} weeks of gestation.

Methods This was a retrospective analysis of the maternal serum free β-hCG and PAPP-A levels in relation to the maternal smoking status in 109 263 chromosomally normal singleton pregnancies that had undergone first-trimester screening for Down syndrome by a combination of fetal nuchal translucency thickness and maternal serum biochemistry.

Results There were 95 287 nonsmokers and 13 976 cigarette smokers. The overall median PAPP-A MoM among cigarette smokers was 0.827, which was 19.6% lower than the value of 1.029 in nonsmokers ($p < 0.0001$ for $\log_{10}$ MoM). The respective values for β-hCG MoM were 1.003 for smokers and 1.035 for nonsmokers ($p < 0.0001$ for $\log_{10}$ MoM) which corresponds to a reduction of 3.1%. There was a significant inverse relationship between the number of cigarettes per day and the level of PAPP-A MoM ($r = 0.989$, $p < 0.0001$) but not the level of free β-hCG MoM ($r = 0.733$; $p = 0.098$). Using a statistical modeling approach we found that the screen-positive rate when correcting the PAPP-A MoM by an all or nil smoking factor was reduced by only 0.1% (3.75 vs 3.85%) when compared to correcting with a factor related to the smoking dose per day.

Conclusion In first-trimester screening for Down syndrome by maternal serum PAPP-A and free β-hCG the impact of correcting for the dose dependant rather than the all or nil effect of smoking is marginal. However, a dose dependent correction improves the accuracy of the individual patient-specific risk. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS: screening; maternal serum biochemistry; PAPP-A; smoking; trisomy; free β-hCG

INTRODUCTION

In trisomy, 21 pregnancies at 11–13^{+6} weeks, the maternal serum concentration of free β-hCG is higher than in chromosomally normal fetuses whereas pregnancy-associated plasma protein A (PAPP-A) is lower (Spencer *et al*., 1999; Canick *et al*., 2006). There is no significant association between fetal nuchal translucency thickness (NT) and maternal serum free β-hCG or PAPP-A in either trisomy 21 or chromosomally normal pregnancies, and therefore the ultrasonographic and biochemical markers can be combined to detect about 90% of affected pregnancies for a false positive rate of 5% (Spencer *et al*., 1999; Nicolaides *et al*., 2005).

In the development of risk algorithms for combined screening the estimation of accurate patient-specific risks necessitates adjustments in the measured free β-hCG and PAPP-A to take into account their temporal variation and association with maternal weight, ethnicity, smoking status, and method of conception (Spencer *et al*., 1999, 2000a,b, 2002, 2003a,c, 2004; Spencer, 2000; Liao *et al*., 2001). Cigarette smoking in the first trimester is associated with changes in the maternal serum concentration of both free β-hCG and PAPP-A. In pregnancies affected by trisomy 21, free β-hCG is reduced by 13% and PAPP-A is increased by about 6% (Spencer *et al*., 2000b) and in chromosomally normal pregnancies, both free β-hCG and PAPP-A are reduced by 3 and about 18% respectively (Spencer, 1999; Spencer *et al*., 2004). In a study of 3779 smokers and 28 951 nonsmokers with chromosomally normal pregnancies, there was a nonsignificant trend towards lower PAPP-A values with increasing cigarette consumption (Spencer *et al*., 2004). Compared to nonsmokers the median PAPP-A was lower by 14.5, 17.6 and 20.1% in those who smoked 3–5, 6–15 and more than 15 cigarettes per day, respectively.

The aim of this study is to examine again on the basis of a much larger number of pregnancies whether there is a significant dose dependency between the number of cigarettes and levels of free β-hCG and PAPP-A at 11–13^{+6} weeks of gestation in chromosomally normal pregnancies. Although a dose-dependant rather than an 'all or nothing' correction for smoking would have little impact at the population level for detection rates, a further reduction in false-positive rates might be achieved and the individual patient-specific risk would

*Correspondence to: K. Spencer, Clinical Biochemistry Department, King George Hospital, Barley Lane, Goodmayes, Essex IG3 8YB, UK. E-mail: KevinSpencer1@aol.com

Received: 8 January 2007
Revised: 26 April 2007
Accepted: 10 May 2007
Published online: 25 June 2007

be more accurate if a dose dependant correction was applied.

METHODS

At Harold Wood Hospital, Essex, and at the Fetal Medicine Centre, London, screening for Down syndrome is carried out by a combination of maternal age, fetal NT, and maternal serum PAPP-A and free β-hCG in a one-stop-clinic for first-trimester assessment of risk (OSCAR) at $11-13^{+6}$ weeks of gestation (Avgidou *et al*., 2005). Transabdominal ultrasound examination is performed to diagnose any major fetal defects and for measurement of the crown-rump length (CRL) and fetal NT (Snijders *et al*., 1998). The Kryptor system (Brahms AG, Berlin) is used to measure PAPP-A and free β-hCG. Maternal demographic characteristics, ultrasononographic measurements and biochemical results are recorded in a computer database. Karyotype results and details on pregnancy outcomes are added into the database as soon as they become available.

Cigarette smoking status at the time of conception is assessed by self-reporting at pretest counselling. In our hands, self-reporting has been shown to be an accurate method of assessing smoking status in pregnant women (Spencer, 1998), while others have shown the same in nonpregnant populations (Vartiainen *et al*., 2002). Others have suggested there is often a significant underreporting of smoking status in pregnant women (Kendrick *et al*., 1995; Boyd *et al*., 1998). This study of 13 976 cigarette smokers and 95 287 nonsmokers is an extension of our previous study of 3779 smokers and 28 951 nonsmokers (Spencer *et al*., 2004) analysing the impact of smoking on first-trimester biochemical markers in chromosomally unaffected singleton pregnancies.

Statistical analysis

In each case, the measured free β-hCG and PAPP-A was converted to maternal weight and gestation corrected MoM using a median regression curve derived previously (Spencer *et al*., 2000a, 2003a). Cases were grouped according to the number of cigarettes smoked per day into 1–2, 3–5, 6–10, 11–15 and more than 15. The cases where the number of cigarettes per day was not recorded and those who reported that they stopped smoking before conception were analyzed separately. Significance of differences in PAPP-A and free β-hCG between the group of smokers and nonsmokers was analyzed by using a t-test for unequal variance on the log MoM. Regression analysis was performed to evaluate the possible dose dependency between the number of cigarettes and the weight-corrected MoMs for PAPP-A and free β-hCG. All statistical analysis were performed using SPSS (SPSS Inc., Chicago, Illinois) and Microsoft Excel (Microsoft Corp., Redmond, Washington).

RESULTS

The search in the two databases identified a total of 109 263 chromosomally normal singleton pregnancies (Fetal Medicine Centre: January 2000–January 2006; Harold Wood Hospital: November 1999–December 2005). At the time of screening the median maternal age was 32.2 (range 16–53) years and the median gestation was 12 ($11-13^{+6}$) weeks. There were 95 287 nonsmokers and 13 976 cigarette smokers, including 139 who reported that they had stopped smoking shortly before conception.

The overall median PAPP-A MoM among cigarette smokers was 0.827, which was 19.6% lower than the value of 1.029 among the nonsmokers ($p < 0.0001$ for $\log_{10}$ MoM). The respective values for β-hCG MoM were 1.003 for smokers and 1.035 for nonsmokers ($p < 0.0001$ for $\log_{10}$ MoM) which corresponds to a reduction of 3.1% (Table 1). Table 2 shows the median MoM values of PAPP-A and β-hCG according to the cigarette consumption per day.

Regression analysis showed a significant inverse relationship between the number of cigarettes per day and the level of PAPP-A MoM (median PAPP-A MoM = 0.769+ (0.152 × 1/number of cigarettes per day), $r = 0.989$, $p < 0.0001$; Figure 1). There was no significant association between the number of cigarettes per day and the level of free β-hCG MoM ($r = 0.733$; $p = 0.098$).

In order to examine the impact on the screen-positive rate of correcting the PAPP-A MoM by a factor related to the smoking dose per day rather than by an all or nil smoking factor, we used a commonly used statistical modeling approach (Royston and Thompson, 1992). The population parameters for the unaffected and Down syndrome groups were as previously described for the biochemical markers (Spencer *et al*., 1999) and the likelihood ratios for unaffected and Down syndrome populations for delta NT were those described in Spencer *et al*., 2003b. From within the Gaussian distributions of $\log_{10}$ free β-hCG and PAPP-A MoMs

Table 1—Differences in free ß-hCG and PAPP-A between nonsmokers and smokers

	Nonsmoker $n = 95\,287$	Cigarette smoker $n = 13\,976$
Median MoM free β-hCG	1.035	1.003
Mean Log10 MoM free β-hCG	0.015064	0.001329 ($p < 0.0001$)
Standard deviation Log10 MoM free β-hCG	0.264411	0.282733
Median MoM PAPP-A	1.029	0.827
Mean Log10 MoM PAPP-A	0.012356	−0.08229 ($p < 0.0001$)
Standard deviation Log10 MoM PAPP-A	0.235943	0.233555

DOI: 10.1002/pd

Table 2—Influence of number of cigarettes smoked per day on median biochemical marker level

Smoker status		Median free β-hCG			Median PAPP-A		
	N	MoM	log10 MoM	t-test	MoM	log10 MoM	t-test
Nonsmoker	95 287	1.035	0.015064	—	1.029	0.012356	—
Smoker 1–2 cigarettes/day	547	1.008	0.00335	$p = 0.302$	0.879	−0.05593	$p < 0.0001$
Smoker 3–5 cigarettes/day	3820	0.991	−0.00378	$p < 0.0001$	0.829	−0.08132	$p < 0.0001$
Smoker 6–10 cigarettes/day	4801	1.004	0.001702	$p < 0.0001$	0.795	−0.09967	$p < 0.0001$
Smoker 11–15 cigarettes/day	425	1.013	0.005604	$p = 0.463$	0.802	−0.09558	$p < 0.0001$
Smoker more than 15 cigarettes/day	288	0.959	−0.01814	$p = 0.033$	0.795	−0.09969	$p < 0.0001$
Smoker–no record of cigarettes/day	3956	1.016	0.007077	$p = 0.067$	0.861	−0.06489	$p < 0.0001$
Smoker–stopped before conception	139	0.965	−0.01531	$p = 0.167$	0.932	−0.03052	$p = 0.032$

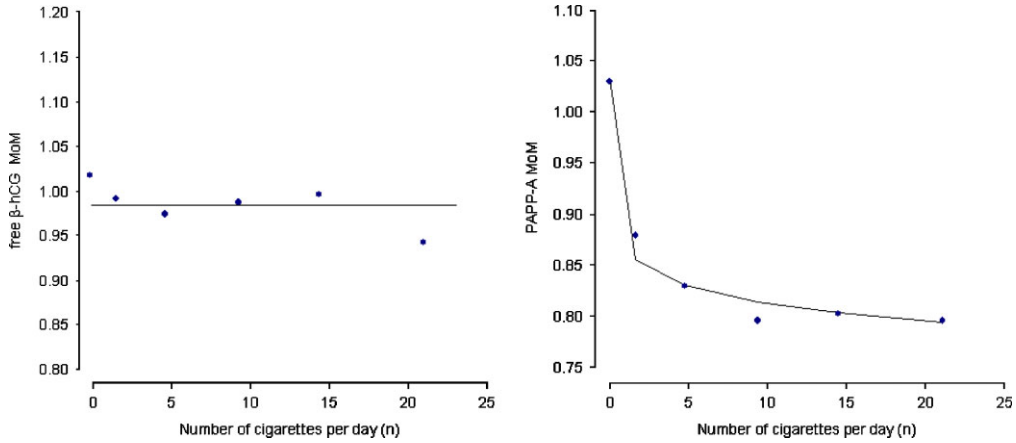

Figure 1—Relationship between maternal cigarette consumption per day and serum level of free β-hCG and PAPP-A

for the unaffected and Down population we created 60 000 random datasets to represent each population. We then reduced at random 12.8% of the PAPP-A results in each group by the all or nil smoking factor of 0.8. We then used each free β-hCG and PAPP-A MoM along with delta NT to calculate a likelihood ratio for Down syndrome and using the *a priori* maternal age risk of Down syndrome from Cuckle *et al.*, 1987, adjusted to risk at 12 weeks of gestation and the maternal age distribution of England and Wales 2000–2002, we calculated a Down Syndrome risk and hence a population screen-positive rate at a risk cutoff of 1 : 300 at time of screening.

To compare the effect of correcting for smoking based on a dose-dependent correction, we took the same corrected PAPP-A result and rather than correction by the all or nil smoking factor we randomly assigned a smoking dose (based on the observed proportion by dose in this study) and corrected the MoM based on the formula described. The simulation was repeated and the screen-positive rate at a risk cutoff of 1 : 300 was compared.

Overall there was only a very marginal change in screen-positive rate of 0.1% when the dose-dependant correction was used (3.75–3.85%).

For example, in a 30-year-old woman who smokes 2 cigarettes per day, first-trimester screening demonstrated a fetal CRL of 60 mm, NT of 2 mm and weight-adjusted PAPP-A of 0.60 MoMs and free β-hCG of 1.80 MoMs. If the maternal serum PAPP-A is adjusted by a factor of 0.8 (the all-or-nil factor) the estimated risk for Down syndrome would be 1 in 2708, compared to 1 in 2251 if the adjustment factor was 0.84 to take into account the number of cigarettes. In effect, for some women, the all or nil correction factor would overcorrect the median PAPP-A.

DISCUSSION

The findings of this study that in chromosomally normal pregnancies at $11–13^{+6}$ weeks of gestation cigarette smoking is associated with a reduction of about 20% in the maternal serum concentration for PAPP-A and 3% in free β-hCG is consistent with the results of previous studies (Spencer, 1999; De Graaf *et al.*, 2000; Niemimaa *et al.*, 2003; Spencer *et al.*, 2004). Additionally, we found an inverse relationship between the number of cigarettes smoked per day and the level of PAPP-A. Even in women who reported that they had stopped smoking shortly before conception, the serum concentration of PAPP-A was 10% lower than in nonsmokers. Unfortunately, the time period between cessation of smoking and conception was not recorded and one has to assume that cessation of smoking was part of a change in lifestyle in view of planned pregnancy.

DOI: 10.1002/pd

Our study and the data interpretation rely upon the accuracy of self-reporting of smoking status by pregnant women–something we have found to be quite reliable in the past in our pregnant population (Spencer 1998). Despite self-reporting having been show to be an accurate indicator of smoking status in non-pregnant individuals (Vartiainen *et al*., 2002) others have raised questions regarding the accuracy of self-reporting amongst pregnant women with a significant underreporting of smoking status during pregnancy of around 14% (Boyd *et al*., 1998). Other studies have shown significant variation in smoking intensity across pregnancy and the reporting of such (Pickett *et al*., 2003). Thus, simple measures of smoking status may lead to under-estimation of the impact of smoking on the fetus or biochemical markers. Even a simple measure of cotinine at the time of screening may be unreliable in establishing the extent of smoking exposure at the time of conception or in the early stages of pregnancy when the biological effects are likely to be just getting established.

The underlying mechanism for the observed decrease in serum concentration of PAPP-A and free β-hCG is uncertain but these proteins are produced by the syncytiotrophoblast and there is evidence that in cigarette smokers there is an increase in synctiotrophoblastic necrosis (Jauniaux and Burton, 1992; Bonno *et al*., 1994; Zdravkovic *et al*., 2005). In the second trimester chromosomally normal pregnancies, the levels of free β-hCG amongst smokers are reduced by 14% (Spencer, 1998). The more pronounced reduction in the second trimester could be either caused by a reduction in release or decreased production of β-hCG in women who smoke. However it is interesting that other placentally derived markers such as inhibin A exhibit markedly increased levels (by 50%) in women who smoke, so alternative mechanisms may be working in addition to syncytiotrophoblast necrosis.

In terms of screening for Down syndrome by first-trimester maternal serum PAPP-A and free β-hCG adjustments should be made to take into account the maternal smoking status. In the overall performance of screening there would be little impact by correcting for the dose-dependant rather than the all or nil effect of smoking. However, the patient-specific risk can be more individualized if the biochemical marker levels are adjusted to the number of cigarettes, which would lead to more accurate risk calculation for the individual. It would appear that the bulk of any impact of smoking on PAPP-A levels is based on relatively light smoking and it would be interesting to assess if passive smoking had a similar impact and to relate these changes to cotinine levels in serum.

ACKNOWLEDGEMENT

This study was supported by a grant from the Fetal Medicine Foundation (Charity No: 1037116) and by a grant from NHS R&D (RF4 Risk Assessment in Pregnancy to KS).

REFERENCES

Avgidou K, Papageorghiou A, Bindra R, Spencer K, Nicolaides KH. 2005. Prospective first-trimester screening for trisomy 21 in 30,564 pregnancies. *Am J Obstet Gynecol* **192**: 1761–1767.

Bonno M, Oxvig C, Kephart GM, *et al*. 1994. Localization of pregnancy-associated plasma protein-A and colocalization of pregnancy-associated plasma protein-A messenger ribonucleic acid and eosinophil granule major basic protein messenger ribonucleic acid in placenta. *Lab Invest* **71**: 560–566.

Boyd NR, Windsor RA, Perkings LL, Lowe JB. 1998. Quality of measurement of smoking status by self-report and saliva cotinine among pregnant women. *Matern Child Health J* **2**: 77–83.

Canick JA, Lambert-Messerlian GM, Palomaki GE, *et al*. 2006. Comparison of serum markers in first-trimester Down syndrome screening. *Obstet Gynecol* **108**: 1192–1199.

Cuckle HS, Wald NJ, Thompson SG. 1987. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. *Br J Obstet Gynaecol* **94**: 387–402.

De Graaf I, Cuckle HS, Pajkrt E, Leschot NJ, Bleker OP, van Lith JM. 2000. Co-variables in first trimester maternal serum screening. *Prenat Diagn* **20**: 186–189.

Jauniaux E, Burton GJ. 1992. The effect of smoking in pregnancy on early placental morphology. *Obstet Gynecol* **79**: 645–648.

Kendrick JS, Zahniser SC, Miller N, *et al*. 1995. Integrating smoking cessation into routine public prenatal care: the Smoking Cessation in Pregnancy project. *Am J Public Health* **85**: 1451–1452.

Liao AW, Heath V, Kametas N, Spencer K, Nicolaides KH. 2001. First-trimester screening for trisomy 21 in singleton pregnancies achieved by assisted reproduction. *Hum Reprod* **16**: 1501–1504.

Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. 2005. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. *Ultrasound Obstet Gynecol* **25**: 221–226.

Niemimaa M, Heinonen S, Seppala M, Ryynanen M. 2003. The influence of smoking on the pregnancy-associated plasma protein A, free beta human chorionic gonadotrophin and nuchal translucency. *BJOG* **110**: 664–667.

Pickett KE, Wakschlag LS, Dai L, Leventhal BL. 2003. Fluctuations of maternal smoking during pregnancy. *Obstet Gynecol* **101**: 140–147.

Royston P, Thompson SG. 1992. Model based screening by risk with application to Down's syndrome. *Stat Med* **11**: 257–268.

Snijders RJM, Noble P, Sebire N, Souka A. 1998. Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. *Lancet* **352**: 343–346.

Spencer K. 1998. The influence of smoking on maternal serum AFP and free beta hCG levels and the impact on screening for Down 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**: 1065–1066.

Spencer K. 2000. Screening for trisomy 21 in twin pregnancies in the first trimester using free beta-hCG and PAPP-A, combined with fetal nuchal translucency thickness. *Prenat Diagn* **20**: 915.

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, Souter V, Tul N, Snijders R, Nicolaides KH. 1999. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* **13**: 231–237.

Spencer K, Ong CY, Liao AW, Nicolaides KH. 2000a. The influence of ethnic origin on first trimester biochemical markers of chromosomal abnormalities. *Prenat Diagn* **20**: 491–494.

Spencer K, Ong CY, Liao AWJ, Papademetriou D, Nicolaides KH. 2000b. First trimester markers of trisomy 21 and the influence of smoking status. *Prenat Diagn* **20**: 852–853.

Spencer K, Crossley JA, Aitken DA, Nix AB, Dunstan FD, Williams K. 2002. Temporal changes in maternal serum biochemical markers of trisomy 21 across the first and second trimester of pregnancy. *Ann Clin Biochem* **39**: 567–568.

Spencer K, Bindra R, Nicolaides KH. 2003a. Maternal weight correction of maternal serum PAPP-A and free beta-hCG MoM when screening for trisomy 21 in the first trimester of pregnancy. *Prenat Diagn* **23**: 851–855.

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

Spencer K, Crossley JA, Aitken DA, Nix AB, Dunstan FD, Williams K. 2003c. The effect of temporal variation in biochemical markers of trisomy 21 across the first and second trimesters of pregnancy on the estimation of individual patient specific risks and the detection rates for Down's syndrome. *Ann Clin Biochem* **40**: 219–231.

Vartiainen E, Seppala T, Lillsunde P, Puska P. 2002. Validation of self reported smoking by serum cotinine measurement in a community based study. *J Epidemiol Community Health* **56**: 167–170.

Zdravkovic T, Genbacev O, McMaster MT, Fisher SJ. 2005. The adverse effects of maternal smoking on the human placenta: a review. *Placenta* **26**(Suppl A): S81–S86.