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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

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Objectives To evaluate the influence of cigarette smoking status on maternal serum free β-hCG, PAPP-A and fetal nuchal translucency (NT) thickness at 11 to 14 weeks of gestation in a large cohort of women screened prospectively for chromosomal anomalies.

Methods Information on maternal cigarette smoking status, maternal age, maternal serum biochemical marker levels and fetal NT were collected from the prenatal screening computer records in two OSCAR screening centres. Data was available from 32 730 unaffected pregnancies and from 124 with Down syndrome. Statistical analysis of the marker levels in the smoking and non-smoking group were carried out. The impact on false-positive rate of correcting for smoking status was assessed from a modelling exercise.

Results Prevalence of smoking was significantly affected by maternal age with an overall incidence of 11.5%, which varied from 35% in women under 20 to 7% in women over 35. In the unaffected population, the median free β-hCG MoM was significantly lower in the smoking group (0.97 vs 1.00) as was that for PAPP-A (0.84 vs 1.02). The standard deviation of the $\log_{10}$ MoM free β-hCG was lower in the smoking group and that for PAPP-A was higher in the smoking group. The difference in median marker levels did not seem to be related to the number of cigarettes smoked per day. In the group with Down syndrome, the median MoM free β-hCG was not significantly different in the smokers (1.69 vs 1.86) as was that for PAPP-A (0.53 vs 0.57). Fetal delta NT was not significantly different in the unaffected smokers (0.11 vs 0.0 mm) or in those with Down syndrome (1.96 vs 2.25 mm). In the smoking group, when screening using maternal serum biochemistry and age alone, the false-positive rate was 6.17%, compared to 4.67% in an age-matched group of non-smokers. Correcting for smoking status by dividing the measured MoM by the median found in the smoking group resulted in the false-positive rate falling to 4.40%. When screening using NT, maternal serum biochemistry and age, the false-positive rate in smokers was 4.48%, which reduced to 3.46% after correction—in line with the 3.76% in the non-smoking group. The impact on detection rate was too small to be accurately measured.

Conclusions The impact of smoking on first-trimester biochemical marker levels does not seem to be dose related. Whilst correcting first-trimester biochemical markers for maternal smoking status has little impact at the population level for detection rates, a considerable reduction in false-positive rate can be achieved, reducing the level to that seen in non-smokers. However, the effect on the individual patient-specific risk can be substantial and could certainly make a difference to the patient's decision on whether to have an invasive test.

KEY WORDS: prenatal screening; nuchal translucency; Down syndrome; trisomy 21; PAPP-A; free β-hCG

INTRODUCTION

In the second trimester of pregnancy, maternal cigarette smoking has been shown in a number of studies to influence the levels of maternal serum biochemical markers used in prenatal screening for Down syndrome. In unaffected pregnancies, smoking is associated with a mean increase in serum AFP (4%) and inhibin A (45–62%), and a decrease in unconjugated oestriol (3%), total hCG (24%) and free β-hCG (14%) (Cuckle *et al*., 1990; Palomaki *et al*., 1993; Bartels *et al*., 1993; Haddow *et al*., 1995; Spencer, 1998; Ferriman *et al*., 1999; Crossley *et al*., 2002; Rudnicka *et al*., 2002). On the whole, the limited data available suggest a similar level of change in pregnancies affected by Down syndrome (Cuckle *et al*., 1990; Spencer, 1998; Crossley *et al*., 2002). In second-trimester screening, correcting for maternal smoking is associated with a less than 1% increase in the overall population detection rate; in four studies, the false-positive rate did not change (Cuckle *et al*., 1990; Palomaki *et al*., 1993; Spencer, 1998; Rudnicka *et al*., 2002), whilst it decreased by 1% in another (Crossley *et al*., 2002).

In first-trimester screening, preliminary data suggest that in cigarette smokers with unaffected pregnancies, PAPP-A is reduced by 15% but free β-hCG is unaltered,

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whereas in pregnancies with Down syndrome, free β-hCG is lower by 13% and PAPP-A is higher by 6% (Spencer, 1999; Spencer *et al*., 2000). These data suggest that if uncorrected these changes would result in a lower detection rate and a higher false-positive rate in cigarette smokers.

The aim of this study was to evaluate the influence of cigarette smoking status on maternal serum free β-hCG, PAPP-A and fetal nuchal translucency (NT) thickness at 11 to 14 weeks of gestation in a large cohort of women.

METHODS

Study population

At Harold Wood Hospital, Essex, and at the Fetal Medicine Centre, London, screening for chromosomal anomalies was carried out in a one-stop (OSCAR) clinic at 11 to 14 weeks using a combination of fetal NT, measured by appropriately trained sonographers, and maternal serum PAPP-A and free β-hCG (Kryptor analytical system, Brahms AG, Berlin). Cigarette smoking status was assessed by self-reporting at pretest counselling. Self-reporting has been shown to be an accurate method of assessing smoking status in pregnant women (Spencer, 1998). All clinical and analytical data were stored in a fetal database (ViewPoint, Webbling, Germany) along with the outcome of pregnancy. The study group comprised unaffected pregnancies from prospective screening studies on a total of 32 730 unaffected and 124 trisomy 21 pregnancies (Bindra *et al*., 2002; Spencer *et al*., 2003a) and from the fourth year of prospective screening at Harold Wood Hospital) for which smoking status was recorded (98% of all cases screened). In 85% of the cases who smoked, information on the amount smoked per day was also recorded.

Data analysis

Analytical results for the biochemical markers from the unaffected pregnancies were converted to weight-corrected MoMs for the respective gestation calculated from the crown-rump length using a median regression curve derived previously and published weight correction formulae (Spencer *et al*., 2003b). Delta NT was similarly derived from the measured nuchal translucency thickness and crown-rump length for the unaffected pregnancies from a previously established relationship (Snijders *et al*., 1998). A recent Fetal Medicine Foundation study has shown that delta NT rather than NT MoM is the most appropriate statistical method of handling NT data (Spencer *et al*., 2003c) in order to produce accurate patient-specific risks. All statistical analysis was performed using Analyse-It (Smart Software, Leeds) and Microsoft Excel.

In order to estimate the impact of correcting for smoking status, data from the 3779 women who smoked were compared with results from a similar number of non-smoking women, matched for maternal age and selected at random from the 28 951 self-reported non-smokers with an unaffected pregnancy. The affected group included the 124 cases with Down syndrome. Detection and false-positive rates were compared before and after correction using biochemical markers alone or biochemical and ultrasound markers in combination.

RESULTS

Women attending the clinic at Harold Wood Hospital are from a general routine obstetric population of a typical District General Hospital. The median age (years) at time of screening of the study population in this centre was 29.97 (range 15–47) with a mean of 29.74 (sd 5.67). The Fetal Medicine Centre is a private centre where women largely self-refer. The median age (years) at time of screening in this study population was 34.0 (range 16–49) with a mean of 33.92 (sd 4.20). The difference in median ages of the population is a result of women at the private centre self-referring because of increased anxiety due to advancing maternal age.

The proportion of women who smoke during pregnancy in the Harold Wood population was 16.9%, this was a slight reduction from the 19% observed in a second-trimester study of women screened between 1991 to 1994 (Spencer, 1998). Patterns of smoking vary considerably with maternal age (Spencer, 1998) and a variety of other socio-economic factors. In the Harold Wood study group, the incidence of smoking by maternal age is shown in Table 1.

The proportion of women who smoke during pregnancy in the Fetal Medicine Centre population was 4.7%, considerably lower than that in the Harold Wood population. In this group of older women of high socio-economic grouping, this could be expected, but again

Table 1—Prevalence of cigarette smoking in the unaffected population according to maternal age

Age band (years)	Harold wood smokers (%)	Fetal medicine centre smokers (%)	Number of non-smokers	Number of smokers	Total	Smokers (%)
<20	39.8	25.5	761	416	1177	35.3
20–24	29.2	16.3	2421	802	3223	24.9
25–29	18.0	5.8	6439	1009	7448	13.5
30–34	12.8	3.8	10 267	882	11 149	7.9
35–39	11.1	4.6	7684	576	8260	7.0
>39	10.7	2.4	1379	94	1473	6.4
All	16.9	4.7	28 951	3779	32 730	11.5

the incidence of smoking was influenced by maternal age (Table 1).

The overall prevalence of cigarette smoking decreased with maternal age (Table 1) and was not significantly different in the trisomy 21 and unaffected pregnancies (8.1% vs 11.5%, $\chi^2 = 1.784$, $p = 0.182$). However, since the incidence of Down syndrome increases with maternal age, this must be taken into account when determining the effect of smoking on the incidence of Down syndrome (Chen *et al*., 1999; Spencer *et al*., 2000; Crossley *et al*., 2002). On the basis of the maternal age distribution of the Down syndrome pregnancies and the prevalence of smoking in the various age bands (Table 1), we would have expected that 13 of the 124 (10.5%) mothers in the Down pregnancies were smokers, rather than the observed 10 (8.1%, $\chi^2 = 0.43$, $p = 0.5$).

In the unaffected pregnancies, the median values of both PAPP-A MoM and free β-hCG MoM were significantly lower in cigarette smokers than non-smokers ($p < 0.0001$, t-test of unequal variance using the $\log_{10}$ MoM). For PAPP-A, the SD of $\log_{10}$ PAPP-A MoM was significantly lower in the smoking group ($p < 0.0001$, variance ratio F-test). For free β-hCG, the SD of $\log_{10}$ free β-hCG MoM was significantly higher in the smoking group ($p < 0.0001$) (Table 2). In the Down syndrome pregnancies, there were no significant differences between cigarette smokers and non-smokers in either PAPP-A MoM ($p = 0.488$) and free β-hCG MoM ($p = 0.075$) or in SD of $\log_{10}$ PAPP-A MoM ($p = 0.152$) and SD of $\log_{10}$ free β-hCG MoM ($p = 0.136$). There was no significant difference in delta NT between cigarette smokers and non-smokers in either the unaffected ($p = 0.098$, z-test) or Down pregnancies ($p = 0.161$).

When the median biochemical marker levels in the unaffected pregnancies were examined according to the number of cigarettes smoked per day, there was no observed relationship between number smoked and the change in median marker MoM (Table 3).

In the management of individual patients, correction for smoking status can be achieved by dividing the weight-corrected MoM in smokers by 0.84 for PAPP-A and 0.97 for free β-hCG. Such correction assumes that the changes in PAPP-A and free β-hCG in smokers in Down syndrome pregnancies are similar to those observed in unaffected pregnancies. The extent to which the observed differences between smokers and non-smokers in the Down pregnancies become significant with an increase in the number of affected pregnancies examined remains to be determined. However, combining the present series of 124 cases with Down syndrome, the 224 published previously (Spencer *et al*., 2000) produces a group of 321 cases from non-smokers and a group of 37 from smokers. The mean delta NT between the smoking (2.457) and non-smoking group (2.639) was not significant ($p = 0.58$, z-test). The median MoM free β-hCG was 2.031 in the non-smokers and 1.884 in the smokers, when compared using t-tests on unequal variance, the log 10 MoM's were not significantly different ($p = 0.281$). The median MoM PAPP-A was 0.595 in the non-smokers and 0.778 in the smokers, when compared using t-tests of unequal variance, the log10 MoMs were also not significantly different ($p = 0.267$).

To assess the impact of correcting for smoking status using these adjustments, we calculated the screen-positive rate from the study of a randomly selected group of 3779 non-smokers, who were matched for maternal age with the cohort of 3779 smokers, and the detection rate from the study of the 124 cases with Down syndrome. We then used the Fetal Medicine Foundation risk algorithm to calculate the risk for Down syndrome for each patient, both by a combination of maternal age, gestational age (calculated from the fetal crown-rump length) and maternal weight-adjusted serum free β-hCG and PAPP-A in MoM, as well as by this combination with fetal NT (Spencer *et al*., 1999). The proportion of Down syndrome and unaffected cases with a risk of greater than 1 in 301 (for combined screening) or greater than 1 in 101 (for biochemical screening alone) was calculated for the smoking and non-smoking groups (Table 3). The false-positive rate in the non-smokers was lower than that in the smokers by about 25% when screening using only biochemical markers and by 16% when screening using biochemical markers and fetal NT. Correcting the biochemical markers for smoking status resulted in the false-positive rate amongst smokers

Table 3—Influence of number of cigarettes smoked per day on median biochemical marker levels

Cigarettes per day	Cases	Median PAPP-A MoM	Median free β-hCG MoM
<3	149	0.792	1.020
3 to 5	1215	0.855	0.949
6 to 10	1530	0.824	0.977
11 to 15	209	0.821	1.009
>15	121	0.799	1.081

Table 2—Marker levels in unaffected and Down pregnancies

	Unaffected			Down syndrome		
	Total	Smokers	Non-smokers	Total	Smokers	Non-smokers
Median PAPP-A (MoM)	1.00	0.84*	1.02	0.56	0.53	0.57
SD of log 10 PAPP-A MoM	0.2349	0.2283	0.2336	0.2655	0.2577	0.2683
Median free β-hCG (MoM)	1.00	0.97*	1.00	1.84	1.69	1.86
SD of $\log_{10}$ free β-hCG MoM	0.2592	0.2803	0.2566	0.2792	0.2545	0.2715
Delta nuchal translucency (mm)	0.0	0.11	0.0	2.23	1.96	2.25
N	32 730	3779	28 951	124	10	114

* $p < 0.0001$

Table 4—Impact of correcting biochemical marker MoMs for smoking status on the detection rate and false-positive rate when screening in the first trimester using either biochemistry alone or in conjunction with fetal NT

	Biochemistry alone (cut-off 1 in 100)		Biochemistry and fetal NT (cut-off 1 in 300)	
Smoking status	False-positive rate (%)	Detection rate (detected/total cases) (%)	False-positive rate (%)	Detection rate (detected/total cases) (%)
Non-smoker	4.67 (176/3779)	64.0 (73/114)	3.76 (142/3779)	89.5 (102/114)
Smoker—uncorrected	6.17 (233/3779)	60.0 (6/10)	4.48 (169/3779)	80.0 (8/10)
Smoker—corrected	4.40 (166/3779)	50.0 (5/10)	3.46 (131/3779)	70.0 (7/10)

falling to a rate similar to that in the non-smokers. In the Down syndrome pregnancies, the numbers of smokers examined ($n = 10$) was too small to identify significant differences in detection rates (Table 4).

DISCUSSION

In the Down syndrome pregnancies, there was a non-significant trend for a lower prevalence of cigarette smoking. This is compatible with the extensively reported negative association between Down syndrome births and maternal cigarette smoking (Kline *et al*., 1981, 1993; Shiono *et al*., 1986; Hook and Cross, 1985, 1988; Christianson and Torfs, 1988; Chen *et al*., 1999) and a deficit of smokers amongst women with pregnancies affected with Down syndrome in our previous study (Spencer *et al*., 2000). However, a recent meta-analysis has challenged such an association (Rudnicka *et al*., 2002).

This study of pregnancies at 11 to 14 weeks of gestation has demonstrated that cigarette smoking is associated with a significant decrease in maternal serum PAPP-A by about 18%, and free β-hCG by about 3%. These findings are similar to those of previous smaller first-trimester studies (Spencer, 1999; De Graaf *et al*., 2000; Spencer *et al*., 2000; Niemimaa *et al*., 2003). In the pregnancies with Down syndrome, there were no significant differences between smokers and non-smokers in either PAPP-A or free β-hCG. It is possible that this finding is a consequence of the small number of smokers examined in this group, but this issue requires further investigation.

In the second trimester, levels of free β-hCG amongst smokers have been shown to be reduced by 14% in unaffected pregnancies and by 16% in cases with trisomy 21 (Spencer, 1998; Ferriman *et al*., 1999). It would therefore seem that continued smoking during pregnancy has an increasing effect on the placenta to bring about reduction in release or production of free β-hCG amongst women who smoke. Further longitudinal studies in women who smoke are required to study this process further. A lack of dose response on marker levels has previously been shown by Bartels *et al*. (1993) for second-trimester markers and this is confirmed for first-trimester markers in this study. This lack of dose response plus the gestational variability of response may suggest that it is not smoking *per se* that has an impact, but it may be something that is closely associated with smoking such as life style, diet or alcohol intake.

The finding of no significant difference in delta NT between cigarette smokers and non-smokers in either the unaffected or Down pregnancies is compatible with our previous data from the study of 2287 non-smokers and 600 smokers in unaffected pregnancies and 197 non-smokers and 27 smokers in pregnancies with Down syndrome (Spencer *et al*., 2000). In contrast, Niemimaa *et al*. (2003) has reported a significantly higher NT MoM in 454 smokers than that in 3825 non-smokers (1.12 vs 1.05).

In screening for Down syndrome by first-trimester serum biochemistry, cigarette smoking is associated with an increase in the false-positive rate. Inevitably, this increase is dependant on the prevalence of smokers and the age distribution of the screened population (Spencer, 1998; Crossley *et al*., 2002; Rudnicka *et al*., 2002). As demonstrated in our study, correcting for smoking status reduces the increased false-positive rate of smokers to that of non-smokers. Additionally, screening for Down syndrome by first-trimester serum biochemistry correction for cigarette smoking can be demonstrated to have a substantial effect on the individual patient-specific risk. For example, in a 30-year-old cigarette smoker with fetal NT of 2.5 mm at a crown-rump length of 64 mm (12 weeks and 4 days of gestation), free β-hCG of 1.01 MoM and PAPP-A of 0.34 MoM, the estimated risk for Down syndrome without correction for smoking is 1 in 207, and this is reduced to 1 in 322 with smoking taken into account. It could be argued that in population screening, adjustment of marker levels for smoking is unnecessary because the prevalence of smokers is low and the effect of smoking is also relatively small (Rudnicka *et al*., 2002). However, the effect on the individual patient-specific risk can be substantial and could certainly make a difference to the patient's decision on whether to have an invasive test or not.

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