

Measurement of nicotine intake in pregnant women —associations to changes in blood cell count

Graham F. Cope, Pamela Nayyar, Roger Holder

Self-reported information about smoking is imprecise and subject to bias, with accuracy varying according to circumstances. Biochemical assessment gives much clearer indications of the effects of tobacco intake on physiological parameters. As part of a randomized controlled trial, a new point-of-care test for smoking was used as a tool to reduce smoking in pregnancy. Measurements of nicotine metabolites in urine were related to a physiological effect of smoking, notably changes to blood parameters, assessed as a routine part of antenatal care. One hundred and eighty-seven pregnant women attending outpatient antenatal care were initially questioned by a midwife about smoking habits and later questioned by the investigators, during which the test was performed and the results relayed back to the patient. Self-reported smoking habit and cigarette consumption, either reported to midwives or the investigators, were shown to be poor indicators of the effects of smoking on blood parameters. The biochemical assessment of nicotine intake was significantly related to white blood count, haemoglobin concentration, haematocrit, mean cell volume, and mean cell haemoglobin. Red cell count, mean cell haemoglobin concentration, and platelet count were unrelated to nicotine metabolite measurements. We concluded that the new test was a reliable measure of nicotine intake in pregnancy and the results correlated with smoking-related changes to haematological parameters.

Introduction

The measurement of biomarkers is a desirable and an increasing means of verifying and quantifying exposure to tobacco smoke (Benowitz, 1996). Self-reported smoking habit is unreliable due to denial and under-reporting, while disclosed cigarette consumption is an imprecise measure of tobacco exposure because of variations in nicotine yield, smoke topography, absorption and metabolism (Perez-Stable, Benowitz, & Marin, 1995). Many biomarkers have been proposed, including expired air carbon monoxide, thiocyanate and nicotine in serum, saliva or urine. However, these markers are marred either by a short half-life or interference from other sources. Nicotine metabolites, notably cotinine, have longer half-lives, on the order of 18 h, and are specific to tobacco

(Benowitz, 1996; Jarvis, Tunstall-Pedoe, Feyerabend, Vesey, & Saloojee, 1987). Many laboratory methods have been developed to measure nicotine metabolites, including gas chromatography (Feyerabend & Russell, 1990), high-performance liquid chromatography (Kyrematen, Damiano, Dvorchik, & Vesell, 1982), radioimmunoassay (Knight, Wylie, Holman, & Haddow, 1985), enzyme-linked immunosorbent assay (Schepers & Walk, 1988) and colorimetric methods (Peach, Ellard, Jenner, & Morris, 1985).

Smoking is an important risk factor for adverse pregnancy outcome, and many women will deny smoking, or under-report cigarette consumption when questioned. Consequently, biochemical measurements are advocated to verify self-report. Kendrick *et al.* (1995) reported that non-disclosure of smoking at enrollment to hospital-based antenatal care was 28%, which rose to 35% at 36 weeks' gestation. Studies of the effects of smoking on pregnancy have found cotinine to be a better predictor of birthweight (Klebanoff, Levine, Clemens, DerSimonian, & Wilkins, 1998; Peacock *et al.*, 1998; Secker-Walker, Vacek, Flynn, & Mead, 1998), and pre-

Graham F. Cope, Pamela Nayyar, Department of Medicine, University of Birmingham; Roger Holder, Department of Mathematics and Statistics, University of Birmingham, Birmingham, UK.

Correspondence to: Graham F. Cope, PhD, Institute of Research and Development, Birmingham Research Park, Vincent Drive, Birmingham B15 2SQ, UK. E-mail: G.Cope.Mermaid@ukonline.co.uk

term and small-for-gestational age deliveries than reported cigarette consumption (Mathews, Smith, Yudkin, & Neil, 1999).

Many of the effects of smoking in pregnancy appear to be related to increased carboxyhaemoglobin concentrations and changes to intravillous blood flow, with interference in the blood supply to the foetus (Merzelina-Roumans, Ubachs, & van Wersch, 1997). Other reports show smoking in pregnancy is associated with changes to maternal blood, notably increased white cell count (Merzelina-Roumans, Ubachs, & van Wersch, 1994; van Buul, Steerers, Jongasma, Eskes, Thomas, & Hein, 1995), mean cell volume, and mean cell haemoglobin concentration (Merzelina-Roumans, Breukers, Ubachs, & van Wersch, 1996). However, many of these findings are equivocal, because they are based on self-reported smoking habit.

The investigation of effective smoking cessation interventions, particularly in pregnancy, is a priority. Point-of-care measurement of smoking, using expired air carbon monoxide monitoring provided biofeedback about baseline smoking, reduced smoking behaviour and improved pregnancy outcome (Secker-Walker, Vacek, Flynn, & Mead, 1997). The drawback of expired air CO measurements is the short half-life and reduced specificity and sensitivity compared to nicotine metabolite measurements (Jarvis *et al.*, 1987). A further study measured serum cotinine by laboratory means and supplied the result to the physician for later feedback to the mother. This approach also reduced smoking with a consequential increase in birthweight, but there was a drawback that the cotinine measurements were retrospective (Haddow, Knight, & Palomaki, 1992).

To overcome the problem of delay with laboratory measurements we developed a point-of-care test to measure nicotine and all its breakdown products in urine by a colorimetric reaction. The 5-min test, based on a colorimetric assay (Peach *et al.*, 1985), enclosed the reagents into a specially designed plastic device (Cope, Nayyar, Holder, Gibbons, & Bunce, 1996). This consisted of a fixed volume syringe and cap and a reaction chamber containing the dried reagents. Unprocessed urine was aspirated by the syringe and introduced onto the reactants, which dissolved, causing the development of a concentration-dependent pink/red derivative in the presence of nicotinic metabolites. The test was quantified by comparison to a cotinine standard by light absorbance at 520 nm and included a compensation factor for urine dilution. This was a measure of the density of the colour of the urine sample prior to reaction and was measured by light absorbance. The results of the test were expressed as cotinine equivalent concentration/urine absorbance ratio. Analysis produced a range of cotinine equivalent concentrations from 0 to 50.9 mg/l and a range of ratio results from 0 to 275. A cut-off value of 10 provided a sensitivity and specificity of 89.9 and 98.9%, respectively. Independent analysis of parallel urine samples

by capillary gas chromatography showed a good correlation between the two methods ($r = 0.89$, $p < 0.001$; Cope *et al.*, 1996).

In a randomized controlled trial we evaluated the test as a tool to increase awareness and improve smoking cessation advice in pregnancy. We used the point-of-care test as part of an interview to provide immediate feedback about nicotine metabolite levels. This was accompanied by an explanation of the significance of the result, with practical advice on how to stop smoking.

The aim of the present study was an observational study to assess how the smoking test results compared to self-reported smoking, both to the investigators and to midwives, with regard to smoking habit and how the test results correlated with physiological effects of smoking in pregnancy, notably changes to maternal blood parameters.

Materials and methods

One hundred and eighty-seven women attending the antenatal clinic of a large inner-city hospital for their initial 'booking' or enrolment visit (between 12 and 16 weeks' gestation) took part in the randomized controlled trial to reduce smoking in pregnancy. As part of their routine screening procedure they were interviewed by a midwife, and a clinical history was taken. This included information about current smoking status and daily cigarette consumption. The women gave a blood sample for routine analysis of a range of parameters including: white cell count, red cell count, haemoglobin concentration, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, and platelet count. The women also provided a urine sample for dipstick screening and bacteriology assessment. It was this sample that was used for nicotine metabolite determination.

Following the screening procedure, all women, smokers and non-smokers alike, following informed verbal consent, were interviewed by the investigators in the knowledge that smoking was being biochemically tested, and were asked detailed questions about current smoking. During this time the smoking test was carried out in the presence of the patient. The colour change and numerical result were relayed back to them with an explanation about the effects of tobacco products on foetal development and potential problems during pregnancy.

The smoking test results and smoking habit reported to both midwife and investigators were later compared with data relating to blood samples obtained from the hospital's pathology department computer database. This information was analysed with Minitab® statistical software. Relationships to smoking status were analysed by two-sample *t*-test and variable parameters by Pearson's correlation coefficients, with $p < 0.05$ taken as statistically significant. Complete haematological profile was compared using MANOVA between different groups of subjects.

Table 1. Value of blood parameters against smoking status determined by biochemical measurement of nicotine metabolite levels in urine

Parameter	Non-smokers ^a	Smokers ^a	<i>p</i> value ^b
<i>n</i>	82	105	
White cell count ($\times 10^3/\mu\text{l}$)	8.4 (2.1)	10.1 (2.9)	$t = -4.61$; $p < 0.0001$
Red cell count ($\times 10^6/\mu\text{l}$)	4.0 (0.4)	3.9 (0.4)	$t = 0.42$; ns
Haemoglobin (g/100 ml)	11.5 (1.1)	11.9 (1.0)	$t = -2.49$; $p < 0.05$
Haematocrit (%)	35.0 (3.1)	36.3 (3.1)	$t = -2.80$; $p < 0.01$
Mean cell volume ($\mu\text{m}^3/\text{red cell}$)	88.7 (6.1)	92.4 (4.5)	$t = -4.50$; $p < 0.0001$
Mean cell haemoglobin (pg/red cell)	29.1 (2.6)	30.2 (1.8)	$t = -3.31$; $p < 0.01$
Mean cell haemoglobin concentration (g/100 ml)	32.7 (1.3)	32.7 (1.0)	$t = 0.41$; ns
Platelet count ($\times 10^3/\mu\text{l}$)	248.0 (55.0)	250.0 (57.0)	$t = -0.17$; ns

^a Two-sample *t*-test. mean (SD) (ns=not significant).

^b Mean (SD).

Results

Self-reported smoking habit reported to the investigators showed the population consisted of 56.1% current smokers, with a daily cigarette consumption (\pm SD) of 12.5(\pm 6.8) cigarettes a day; and 43.9% long-term ex- and non-smokers. Of the self-reported smokers 1.7% had a negative smoking test result. These were all women who reported smoking ≤ 5 cigarettes a day. This gives a sensitivity of 98.3%. The smoking test results (as cotinine equivalent concentration/urine absorbance ratio) was significantly correlated with different categories of daily cigarette consumption reported to the investigators ($r = 0.67$, $p < 0.0001$). The wide spread of results observed in each category represented the variability in smoking behaviour mentioned above, and also the timing of the last cigarette.

The self-reported smoking habit reported to the midwives was different from that reported to the investigators, with denial amounting to 10.3%, and a further 38.4% significantly under-reported their cigarette consumption, leaving 51.3% reporting the same to both parties.

Smoking status (Yes or No) reported to midwives did not correlate significantly with any of the blood parameter measurements. However, cigarette consumption was significantly and positively associated with white blood count ($r = 0.26$, $p < 0.05$), with the association to other parameters failing to reach significance. Smoking status reported to the investigators was strongly associated with white blood count ($t = -4.76$) and mean cell volume ($t = -4.50$, both $p < 0.001$), and significantly associated with haematocrit ($t = -2.72$, $p < 0.01$), mean cell haemoglobin ($t = -3.31$, $p < 0.02$), and with haemoglobin concentration ($t = -2.38$, $p < 0.05$). However, cigarette consumption reported to investigators failed to be associated with any of the parameters.

Smoking status and nicotine intake, as determined by the biochemical test (Table 1), showed strong correlations with white blood count ($t = -4.61$, $p < 0.0001$; $r = 0.25$, $p < 0.001$ respectively), and mean cell volume ($t = -4.50$, $p < 0.0001$; $r = 0.20$, $p < 0.01$ respectively). While haemoglobin, haematocrit and mean cell haemoglobin

were all significantly correlated ($p < 0.05$), red blood count, mean cell haemoglobin concentration, and platelets failed to be associated with smoking, either by self-report or biochemical measurement. Comparison of whole haematological profile by smoking status as determined with the biochemical test and by self-reported smoking status to the investigators showed highly significant differences ($F_{8,178} = 7.16$, $p < 0.0001$ and $F_{8,179} = 7.16$, $p < 0.0001$ respectively).

Discussion

The validity of self-reported smoking habit and cigarette consumption has been called into question for some time, yet many clinicians still rely on this information on which to base important clinical decisions. Research has shown that the accuracy of results obtained with respect to smoking habit will depend on the degree of pressure the smoker feels under not to smoke. For example, self-completed questionnaires for research purposes will provide more reliable results than from interviewer-completed questionnaires, while the setting and personnel asking the questions will alter their reliability (Ford, Tappin, Schluter, & Wild, 1997; Schofield & Hill, 1999). These variables, combined with guilt-laden denial, necessitate that biochemical verification and measurement of nicotine intake have become a requisite for tobacco-related research.

In the present study we have further demonstrated that self-reported smoking habit and cigarette consumption vary with circumstances, and improve in the knowledge that biochemical verification is being undertaken. However, we found that recall of cigarette consumption did not provide information that was as accurate as biochemical measurements. This may be because, in this instance, there is recall bias with possible over-estimation of cigarette consumption.

The biochemical measurements of nicotine metabolites in urine showed that nicotine intake in women in the early stages of pregnancy is highly correlated with an increase in white blood cell count and to a lesser extent with mean cell volume, haemoglobin concentration, haematocrit, and mean cell haemoglobin. The use of

nicotine replacement therapy, without cigarette smoking, has been shown to be correlated with higher white blood count count, suggesting nicotine may be the responsible factor (Jensen, Pedersen, Frederiksen, & Dahl, 1998). Changes to mean cell volume and haemoglobin concentrations may affect oxygen carrying capacity of maternal blood compared to non-smokers and this may have an effect of reducing the efficiency of the placenta and its nutrient-transporting capabilities.

In conclusion we have reiterated the findings that self-reported smoking habit in pregnancy is unreliable and inaccurate; however, reliability does depend on how much pressure the women feels under not to smoke. The biochemical measurements of smoking habit have confirmed that smoking further increases white blood cell count in pregnancy and strengthens previous findings that smoking also increases mean cell volume, haemoglobin, and mean cell haemoglobin concentration, with the effects being dose dependent on nicotine intake. We reiterate the recommendation that, as pressure grows for women not to smoke during pregnancy and as self-deception rates increase, smoking habit in pregnancy should be confirmed with biochemical measurements, preferably by point-of-care testing.

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