Oral Diseases (2004) 10, 287–293

http://www.blackwellmunksgaard.com

Clinical Biochemistry

The validation of self-reported smoking status by analysing cotinine levels in stimulated and unstimulated saliva, serum and urine

V Binnie[1], S McHugh[1], L Macpherson[1], B Borland[2], K Moir[3], K Malik[4]

[1]*Glasgow Dental School, Glasgow, UK;* [2]*Department of Biochemistry, North Glasgow NHS Trust, Gartnavel General Hospital, Glasgow, UK;* [3]*Royal Dental Hospital of Melbourne, Melbourne, Australia;* [4]*Birmingham Dental School, Birmingham, UK*

OBJECTIVES: Cotinine, a nicotine metabolite, can be used to measure exposure to tobacco smoke. The aim of this study was to compare cotinine levels in different biological fluids collected from both smokers and non-smokers and to relate the findings to self-reported smoking status. Data were also collected concerning the acceptability of the differing methods of sample collection.

MATERIAL AND METHOD: Patients recruited to the study were asked to provide samples of urine, blood and saliva (both stimulated and unstimulated). Data collected from patients by questionnaire included information on smoking behaviour such as daily number of cigarettes and environmental exposure to smoke. After the sample collection, patients were asked to rate the acceptability of each sampling method. Samples were analysed using enzyme immunoassay (EIA) kits.

RESULTS: In total, 80 patients participated, with 49 being smokers and 31 being non-smokers. There was clear differentiation between smokers and non-smokers ($P < 0.001$) for all the different samples in terms of cotinine. A significant relationship was seen between cotinine and daily number of cigarettes for both salivas and urine (all $P < 0.001$) but not for serum. Participants found serum and urine collection methodologies 'very acceptable' (67 and 66%, respectively) whereas 9% found collection of stimulated saliva 'not at all acceptable'.

CONCLUSION: Cotinine, whatever the collection method and analysed by EIA kits, shows good differentiation between smokers and non-smokers. Salivary samples have the advantage of being non-invasive, although collection methodology is important, as cotinine levels may vary.

Oral Diseases (2004) **10**, 287–293

Correspondence: V Binnie, Level 8, Glasgow Dental School, 378 Sauchiehall Street, Glasgow G2 3JZ, UK. Tel: 0141-211-9802; Fax: 0141-211-9776, E-mail: v.binnie@dental.gla.ac.uk
Received 1 October 2003; revised 16 February 2004; accepted 12 March 2004

Keywords: tobacco; cotinine; cross-sectional study

Introduction

Tobacco use is the single biggest contributor to ill health, and is the most important preventable cause of death in the UK (Callum, 1998). There is growing awareness and interest in the role that dental health professionals can play in helping their patients quit the tobacco habit, whether in secondary or primary care (Chestnutt and Binnie, 1995; McCann *et al*, 2000; Warnakulasuriya 2002; Watt and Daly, 2003).

In addition to being implicated in coronary health disease, lung and other cancers, smoking also has a profound effect on the oral tissues. Cigarette smoking is associated with increased prevalence and severity of periodontitis and smokers suffer from more tooth loss (Krall *et al*, 1997; Tonetti, 1998). In addition, the risk of oral cancer and potentially malignant lesions is higher amongst smokers compared with those who have never smoked. Patients who smoke have a sixfold increased risk of developing oral leukoplakia compared with non-smokers (Baric *et al*, 1982). There is some evidence that if patients with such lesions can be encouraged to quit the tobacco habit, such lesions will regress (Gupta *et al*, 1986; Chad Martin *et al*, 1999). However, neither of these studies used biochemical validation to monitor changes in tobacco exposure.

When evaluating the effectiveness of tobacco cessation advice, it is important that some form of biochemical validation is used. The most commonly used means of evaluating tobacco exposure is the measurement of carbon monoxide in an exhaled air sample. Although advantages of this method include cost and ease of use, disadvantages include non-specificity and a short half-life of 3–6 h, which can lead to false negatives. One biochemical marker that is able to determine exposure to tobacco smoke over a longer timeframe, with a half-life of 20 h, is cotinine. This compound is a metabolite of nicotine and can be measured in a number of

biological fluids including blood, saliva, cervical exudate, semen and urine (Etzel, 1990; Vine *et al*, 1993; Poppe *et al*, 1995). Cotinine is sufficiently sensitive to be detected also in the body fluids of those individuals exposed to passive or environmental tobacco smoke (Cummings *et al*, 1990).

Most studies using cotinine assays have relied on serum samples, which can be problematic in field settings. Urine is non-invasive to collect, but requires access to facilities for its collection.

Salivary samples, taken for use in cotinine analysis, also have the benefit of being non-invasive, and have been shown to be stable if sent by post, thus enabling their use in outreach studies (Greeley *et al*, 1992; Smith *et al*, 1998). Salivary cotinine has also been used extensively to determine exposure to smoke in large population studies, such as the health surveys in Scotland and England (Shaw *et al*, 2000; Bajekal *et al*, 2003).

Most previous studies using saliva have failed to specify exactly how the saliva is collected although it has been suggested that levels of cotinine can vary depending on whether the saliva collected is stimulated or unstimulated. However, one study has examined the relationship between cotinine levels and collection method and found that stimulated samples had lower levels of cotinine than those found in unstimulated saliva (Schneider *et al*, 1997).

Traditionally, cotinine has been measured using a number of techniques including radioimmunoassay, gas liquid chromatography or liquid chromatography (Feyerabend and Russell, 1990). However, more recently, a microplate enzyme immunoassay (EIA) has become available in the UK. An advantage of these kits is that large, expensive equipment is not required, but to date the kits are relatively unevaluated in the UK.

With regards to the patient's perspective, no published work has previously investigated patient acceptability of the different sampling methods used to collect biological samples for cotinine analyses.

The main aim of this study was to measure and compare cotinine levels using the microplate EIA technique in a variety of biological fluids, collected from a group of patients, both smokers and non-smokers, recruited in an outpatient oral medicine department. Further aims were to:

(a) Correlate self-reported smoking exposure data with the biochemical determination of cotinine levels in the various body fluids;
(b) Compare the patient acceptability of the differing methods of sample collection.

Material and method

Following approval from the Greater Glasgow Area Dental Ethics Committee, recruitment was via patients attending the oral medicine outpatient clinic at Glasgow Dental Hospital and School. Initially, smokers were invited to participate and non-smokers were then recruited, in an attempt to match age and gender to the case group. Data were collected over a 3-month period in early summer, by two student researchers and one academic researcher. A convenience sample was used, with as many patients recruited as possible within the available timeframe.

The age range for the participants was 16–75 years. Exclusion criteria included medical conditions such as an incipient diagnosis of oral carcinoma or medication affecting salivary function. For smokers, only those who used cigarettes were included in the study: those individuals who smoked a pipe or used cigars were excluded, as were any individuals currently using nicotine replacement therapy.

Participating patients were asked to fill in a questionnaire about their tobacco smoke exposure. The questionnaire sought information on daily number of cigarettes smoked, time of first cigarette of the day, inhalational habits and brand and tar levels of current cigarettes used. The time elapsed since the most recent smoking occasion was also noted.

For non-smokers, information on exposure to tobacco smoke both at home and in the workplace was collected. Additionally, details relating to tobacco smoke exposure in the last 24 h was recorded.

Samples were then collected from each patient in the following order:

1. An unstimulated sample of saliva was collected by asking the patient to drool into a universal container (minimum volume = 3 ml).
2. A sample of stimulated saliva was collected by asking the patient to chew the cotton wool roll from a Salivette collection device (Sarstedt Aktiengesellschaft & Co., Numbrecht, Germany). When saturated, this was removed from the mouth and placed into the salivette.
3. A sample of blood (5 ml) was collected in a plain container using standard venepuncture techniques.
4. A sample of urine (25 ml) was collected in a plain universal container.

Patients were then asked to fill in a short questionnaire concerning the acceptability of the four different methods of sample collection. A four-point Likert scale was used which asked the respondents to rate the sample collection from 'completely unacceptable' to 'completely acceptable'.

Blood samples were stored in a fridge overnight, to allow clotting before the serum was separated and stored at −20°C. Stimulated saliva samples collected using the Salivette devices were centrifuged at 1200 ***g*** for 10 min. The supernatant was then removed and stored at −20°C. The unstimulated saliva and urine samples were stored at −20°C. On the day of analysis, the unstimulated saliva was thawed and centrifuged and the supernatant transferred to inert plastic tubes.

Cotinine concentrations were measured using a microplate EIA (Cozart Biosciences Ltd, Abingdon, UK). Different versions of the assay, with appropriate standards, are available for each of the three biological matrices. Quality control material was prepared by spiking cotinine-free serum, urine and saliva with cotinine standard (Sigma Chemicals) to give two levels, low

and high within each standard range for each matrix. Where required, dilutions of the patients' samples were made using cotinine-free serum, urine and in the case of saliva, deionized water. Serum and salivary cotinine concentrations are expressed as ng ml^{-1}. Urine creatinine was measured by the Olympus kinetic Jaffe reaction on an Olympus 640 analyser (Olympus UK Ltd, Southall, UK). To take account of urine dilution, all urine cotinine results were expressed as a ratio (ng $mmol^{-1}$ creatinine).

A cut-off of 15 ng ml^{-1} of cotinine was used to differentiate smokers from non-smokers in serum and saliva, whilst for the urine sample analysis, a cut-off of 50 ng $mmol^{-1}$ of cotinine was used to determine smokers (SRNT Subcommittee on Biochemical Verification, 2002).

Statistical analysis

For smokers and non-smokers separately, the cotinine levels were approximately normally distributed. When comparing smokers and non-smokers, it was necessary to logarithmically transform the data, due to greatly differing variances in the cotinine levels. Subsequent analysis was performed on the transformed data and reported confidence intervals to compare smokers and non-smokers are for the ratios of the geometric means. Similar analysis was required when comparing non-smokers who were exposed/not exposed to smoke.

For smokers and non-smokers separately, a repeated measures analysis of variance was used to determine whether there were any significant differences between the four collection methods in terms of mean cotinine levels. Subsequent follow-up multiple comparisons were carried out to identify which methods differed significantly. Generalized linear models were used to identify which self-reported factors, for smokers and non-smokers separately, had a significant effect on the cotinine levels, again with suitable follow-up multiple comparisons where necessary.

Results

In total, 80 patients were recruited. Of the participants, 49 (25 male, 24 female) were smokers (61%) and 31 (15 male, 16 female) were non-smokers (39%). The mean age of the smokers was 44 years (s.d. 18 years) and for the non-smokers 47 years (s.d. 17 years). Thirty-eight (48%) of the participants were from relatively affluent backgrounds, i.e. residing in DEPCAT 1–4 areas (21 smokers and 17 non-smokers).

Comparison of smokers vs non-smokers

The mean cotinine level for the four fluids, for smokers and non-smokers separately, is shown in Table 1, together with the 95% confidence interval for the ratio of the geometric mean cotinine levels (smokers/non-smokers). Corresponding *P*-values from the two-sample *t*-tests of equal mean levels of cotinine in smokers and non-smokers are also given.

A clear differentiation between the smokers and non-smokers was seen (with $P < 0.001$ for all fluids) with confidence intervals illustrating the much greater mean cotinine level of smokers. The mean level of cotinine in non-smokers in all the fluids was below 10 ng ml^{-1}, whereas the mean level of cotinine in the smokers varied from 194 ng ml^{-1} in stimulated saliva to 328 ng ml^{-1} in serum.

Table 1 Mean levels of cotinine for smokers and non-smokers for each sampling method

	Smokers ($n = 49$), mean (s.d.)	*Non-smokers* ($n = 31$), mean (s.d.)	*95% CI*[a] *(smokers/non-smokers)*	*P*-value
Serum (ng ml^{-1})	328.4 (207.5)	3.6 (2.8)	59.7–111.7	< 0.001
Stimulated saliva (ng ml^{-1})	194.3 (122.5)	2.0 (0.9)	28.5–105.4	< 0.001
Unstimulated saliva (ng ml^{-1})	314.0 (171.9)	1.6 (1.2)	120.8–235.5	< 0.001
Urine (ng $mmol^{-1}$)	302.5 (244.0)	1.1 (1.9)	224.9–776.3	< 0.001

[a]CI is for ratio of geometric mean of smokers to geometric mean of non-smokers.

Comparison of collection methods

Repeated measures ANOVA indicated that there were significant differences in the mean cotinine levels between the four collection methods, for both smokers and non-smokers (both $P < 0.001$). Subsequent multiple comparisons indicated that for smokers the cotinine levels in serum, urine and unstimulated saliva were significantly greater on average than the levels found in stimulated saliva (Table 2). For non-smokers, where cotinine is being measured in very small amounts, there were significant differences between the serum and all other types of sample, with the mean serum level being significantly higher. When comparing the urine and stimulated saliva in non-smokers, the mean level in the stimulated saliva was significantly higher. This last finding is the opposite relationship to that found with these two samples in smokers, where the mean level of cotinine in urine is higher than that found in the stimulated saliva.

Relationships between self-reported data and cotinine levels for smokers

Results from generalized linear models, incorporating number of cigarettes smoked per day (< 10, 10–20 or

Table 2 Multiple comparisons of sampling methods for smokers and non-smokers

	95% Simultaneous CI for difference in average cotinine levels	
Fluid–fluid	*Smokers* ($n = 49$)	*Non-smokers* ($n = 31$)
Serum–stimulated saliva	59.7–217.7	0.9–2.5
Serum–unstimulated saliva	−63.7–93.2	1.2–2.8
Serum–urine	−52.0–103.9	1.8–3.4
Urine–stimulated saliva	33.7–191.8	−1.7–−0.1
Urine–unstimulated saliva	−89.7–67.3	−1.3–0.3
Unstimulated saliva–stimulated saliva	44.4–203.5	−1.2–0.4

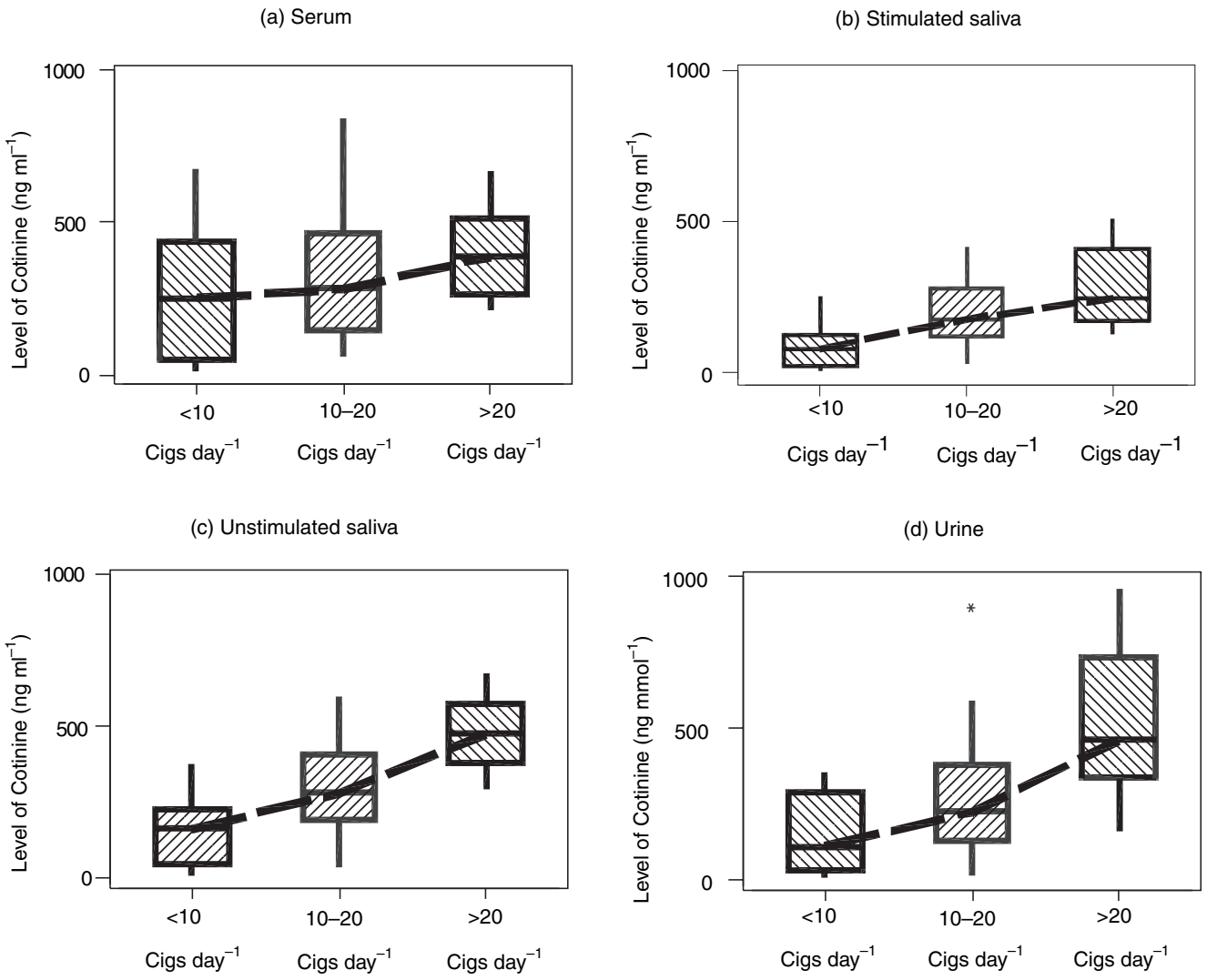

Figure 1 Boxplots of levels of cotinine by number of cigarettes smoked per day for each collection method. Boxplots illustrating dose–response relationship between cotinine and number of cigarettes smoked per day (median trace of cotinine level across NOCS)

>20), tar level of cigarettes smoked (low, medium or high) and inhalational habits (slightly, moderately or deeply) for each of the collection methods separately, suggested that the only factor having a significant influence on the smokers' cotinine levels was number of cigarettes smoked per day (NOCS). The boxplots shown in Figure 1 show a cotinine dose–response relationship with NOCS. This dose–response relationship between cotinine level and NOCS was statistically significant for stimulated saliva (Figure 1b), unstimulated saliva (Figure 1c) and urine (Figure 1d) (all $P < 0.001$) but was not statistically significant for serum ($P = 0.291$) (Figure 1a).

For stimulated saliva, the mean cotinine level for <10 cigarettes day^{-1} was significantly lower than that for 10–20 and >20 cigarettes day^{-1}, but there was no significant difference between the mean cotinine level for 10–20 and that for >20 cigarettes day^{-1}. For unstimulated saliva, there was a significant difference between each of the three categories of NOCS. For urine, there was no significant difference between the average levels of cotinine for <10 and 10–20 cigarettes day^{-1}, but both of these had on average lower levels of cotinine than the >20 cigarettes day^{-1} category.

Relationships between self-reported data and cotinine levels for non-smokers

For non-smokers, generalized linear models were used to determine which of the three factors – exposed to passive smoke at home (yes or no), at work (yes or no) and exposed to passive smoke in the previous 24 h (yes or no) – had a significant influence on the mean cotinine level, for each of the four collection methods in turn (see Table 3).

For each of the fluids, the most dominant factor was exposure to smoke at home. This factor had a significant effect on the average cotinine levels of stimulated saliva, unstimulated saliva and urine (all $P < 0.05$). For each of these fluids exposure to smoke at home significantly increased on average the mean level of cotinine,

Table 3 Mean levels of cotinine for non-smokers for each sampling method by exposure to smoke at home

	Exposed to smoke at home ($n = 10$), mean (s.d.)	*Not exposed to smoke at home* ($n = 21$), mean (s.d.)	*95% CI*[a] *(exposed/ not exposed)*	*P*-value
Serum (ng ml^{-1})	5.4 (4.7)	2.9 (0.8)	0.4–1.4	0.25
Stimulated saliva (ng ml^{-1})	2.7 (1.4)	1.7 (0.4)	0.5–0.9	0.02
Unstimulated saliva (ng ml^{-1})	2.5 (1.9)	1.3 (0.4)	0.4–1.0	0.04
Urine (ng mmol^{-1})	2.3 (2.0)	0.6 (1.5)	0.1–0.5	<0.01

[a]CI is for ratio of geometric mean of exposed to geometric mean of not exposed.

compared with the mean level of cotinine of participants not exposed to smoke at home.

Patient acceptability of each collection method

Figure 2 gives the percentages of patients who found each of the four collection methods 'very acceptable', 'moderately acceptable', 'tolerable' and 'not at all acceptable'. There was no difference between the patterns of acceptability of smokers and non-smokers. High numbers of patients found both serum and urine collection methodologies 'very acceptable' (67 and 66%, respectively), significantly greater than that for the stimulated saliva collection (45%). No participant rated the serum or urine collection methods as being 'not at all acceptable', whilst almost one in 10 (9%) found collection of the stimulated saliva 'not at all acceptable'. The unstimulated saliva fared better than the stimulated saliva, with 51% of the participants rating the former collection method as 'very acceptable' and only 1% 'not at all acceptable'.

Discussion

For monitoring purposes within a smoking cessation trial, usually only one type of biological sample would be collected. Choice of sample type depends upon the sampling means available and the setting in which the sample is collected.

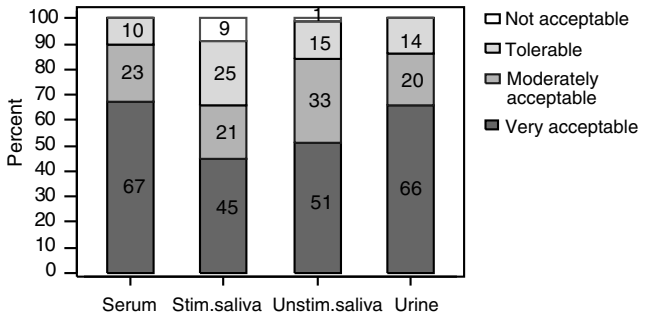

Figure 2 Barcharts of patient acceptability for each collection method. Percentage of patients finding serum, stimulated saliva, unstimulated saliva and urine collection methods 'very acceptable', 'acceptable', 'moderately acceptable', and 'not at all acceptable'

This study aimed to compare cotinine levels and patient acceptability in four different collection methods within an oral medicine setting. It is acknowledged that the utilization of a convenience sample may have resulted in participants not being necessarily representative of the typical oral medicine patient population. Although a reasonable number of participants were recruited, the matching of non-smokers and smokers was incomplete due to time constraints, resulting in an unequal number of patients in the two groups.

In this study, using the appropriate microplate EIA kit for each type of sample, good differentiation was obtained between smokers and non-smokers. Levels of cotinine varied among the different samples collected in the study. The half-life of cotinine in saliva and serum is approximately the same, and cotinine concentrations in these two matrices have previously been found to be correlated. Bernert *et al* (2000) reported that levels of cotinine in salivary samples (collected via salivette devices) were predictive of serum cotinine levels by $\pm 10\%$. However, in the present study, serum levels were found to be more closely correlated with unstimulated than stimulated saliva: for smokers the serum cotinine was on average 4 and 41% greater than the unstimulated and stimulated salivas, respectively.

In this study, the mean level of cotinine in smokers was found to be greater in the unstimulated (314 ng ml^{-1}) compared with the stimulated (194 ng ml^{-1}) saliva. These findings were consistent with those of Schneider *et al* (1997), who postulated that the reason for the difference may lie in the pH changes which alter with the flow rate. Cotinine has a pK_a close to the pH of saliva and plasma. As the pH of unstimulated saliva is less than that of stimulated saliva, a basic compound such as cotinine would be influenced by the flow. Under more acidic conditions such as those produced by unstimulated saliva, there would be higher concentrations available of cotinine. Thus, as flow rate is increased with stimulation, less of the substance would be captured for measurement.

A cotinine dose–response relationship to nicotine exposure is important as it helps to quantify the relative risk that patients are undergoing. In this study, the two salivary samples and urine samples were able to differentiate between categories of light, medium and heavy smoker, whereas serum samples were not able to exhibit this finding. Machacek and Jiang (1986) found similar findings, with poor correlation between cotinine concentrations in plasma and number of cigarettes smoked. With regards to saliva and cotinine levels, Etter *et al* (2000) found cotinine concentration to be moderately associated with the number of cigarettes per day.

Given the reported relationship between cotinine concentrations and cigarettes per day, it may be possible to use this analysis in longitudinal studies to differentiate between smokers who report they have reduced smoking but who continue to compensatory smoke by inhaling more deeply (i.e. cotinine levels will be maintained), and smokers who actually cut down smoking exposure prior to quitting totally. This is an area requiring further investigation.

Patient acceptability of the different methods of sample production provided some unexpected findings. It was postulated that patients might rate venepuncture least favourably because of the invasive nature of the technique. However, surprisingly they rated the stimulated saliva collection most negatively. Some participants found the chewing of the cotton wool roll an unpleasant sensation and, in extreme cases, felt nauseous, which may have led to a poor acceptability rating being recorded. From a participant's point of view, the unstimulated saliva appeared to be the more acceptable of the two salivary collection methods, and hence would be the choice for any future work within a dental setting. Acceptability levels associated with alternative means of stimulating saliva would require further investigation.

The high acceptability of the blood sampling methodology may be related to the setting in which this cross-sectional study took place: a dental hospital oral medicine department where venepuncture is often a routine part of investigation. It is postulated that work carried out in a different dental setting such as general dental practice or a periodontal clinical may yield differing results in terms of patient acceptability of collection methods.

It is important that any future smoking cessation interventions within a dental setting are monitored, and that quit rates are biochemically verified. One study which took place in a hospital periodontal department, found a difference in quit rates between intervention and control groups of 7%. However, this information was gathered by self-report (Macgregor, 1996). The first UK study of smoking cessation in general dental practice did use cotinine to biochemically verify those participants who reported that they had quit smoking and had a success rate of 11% (Smith *et al*, 1998). Whole mouth salivary samples were collected for cotinine analysis by gas chromatography.

For future smoking cessation work, baseline verification of cotinine levels, followed by cotinine assessment once smoking cessation interventions have taken place offer a good means of monitoring and evaluating the process of smoking cessation interventions as they are delivered. For patient motivation and feedback, if this is combined with a more immediate means of determining exposure, similar to that obtained with an exhaled air sample measured using a carbon monoxide monitor, the greater may be the benefit for the patients (Murray *et al*, 2002).

In conclusion, the results of this study show that, with the use of EIA kits, any of the four collection methods would be appropriate for biochemical validation of tobacco exposure. From a practical perspective, saliva would be the most appropriate for use by oral health staff, and patients' opinions would suggest using an unstimulated rather than stimulated collection method.

References

Bajekal M, Primatesta P, Prior G (2003). *Health survey for England, 2001*. The Stationery Office: London.

Baric JM, Alman JE, Feldman RS, Chancey HH (1982). Influence of cigarette smoking, removable partial dentures and age on oral leucoplakia. *Oral Surg Oral Med Oral Pathol* **54:** 424–429.

Bernert JT, McGuffey JE, Morrison MA, Pirkle JL (2000). Comparison of serum and salivary cotinine measurements by sensitive high-performance liquid chromatography – tandem mass spectrometry method as an indicator of exposure to tobacco smoke among smokers and nonsmokers. *J Anal Toxicol* **24:** 333–339.

Callum C (1998). *The UK smoking epidemic: deaths in 1995*. Health Education Authority: London.

Chestnutt IG, Binnie VI (1995). Smoking cessation counselling – a role for the dental profession? *Br Dent J* **179:** 411–415.

Cummings KM, Markello SJ, Mahoney M, Bhargava AK, McElroy PD, Marshall JR (1990). Measurement of current exposure to environmental tobacco smoke. *Arch Environ Health* **45:** 74–79.

Etter JF, Vu DT, Perneger TV (2000). Saliva cotinine levels in smokers and nonsmokers. *Am J Epidemiol* **151:** 251–258.

Etzel RA (1990). A review of the use of saliva cotinine as a marker of tobacco smoke exposure. *Prev Med* **19:** 190–197.

Feyerabend C, Russell MA (1990). A rapid gas–liquid chromatographic method for the determination of cotinine and nicotine in biological fluids. *J Pharm Pharmacol* **42:** 450–452.

Greeley DA, Valois RF, Bernstein DA (1992). Stability of salivary cotinine sent through the U.S. mail for verification of smoking status. *Addict Behav* **17:** 291–296.

Gupta PC, Mehta FS, Pindborg JJ *et al* (1986). Intervention study for primary prevention of oral cancer among 36 000 Indian tobacco users. *Lancet* **21:** 1235–9.

Krall EA, Dawson-Hughes B, Garvey AJ, Garcia RI (1997). Smoking, smoking cessation, and tooth loss. *J Dent Res* **76:** 1653–1659.

Macgregor ID (1996). Efficacy of dental health advice as an aid to reducing cigarette smoking. *Brit Dent J* **180:** 292–296.

Machacek DA, Jiang NS (1986). Quantification of cotinine in plasma and saliva by liquid chromatography. *Clin Chem* **32:** 979–982.

Martin CG, Brown JP, Eifler CW, Houston GD (1999). Oral leucoplakia status six weeks after cessation of smokeless tobacco. *J Am Dent Assoc* **130:** 945–54.

McCann MF, Macpherson LM, Binnie VI, Stephen KW (2000). A survey of primary care dental practitioners' oral cancer-related practices and training requirements. *Comm Dent Health* **17:** 24–30.

Murray RP, Connett JE, Istvan JA, Nides MA, Rempel-Rossum S (2002). Relationship of cotinine and carbon monoxide to self-reported smoking in a cohort of smokers and ex-smokers followed over 5 years. *Nicotine Tob Res* **4:** 287–294.

Poppe WA, Peeters R, Daenens P, Ide PS, Van Assche FA (1995). Tobacco smoking and the uterine cervix: cotinine in blood, urine and cervical fluid. *Gynecol Obstet Invest* **39:**110–114.

Schneider NG, Jacob P III, Nilsson F, Leischow SJ, Benowitz NL, Olmstead RE (1997). Saliva cotinine levels as a function of collection method. *Addiction* **92:** 347–351.

Shaw A, McMunn A, Field J (2000). *Scottish health survey 1998*. The Stationery Office: Edinburgh.

Smith SE, Warnakulasuriya KA, Feyerband C, Belcher M, Cooper DJ, Johnson NW (1998). A smoking cessation programme conducted through dental practices in the UK. *Br Dent J* **185:** 299–303.

SRNT Subcommittee on Biochemical Verification (2002). Biochemical verification of tobacco use and cessation. *Nicotine Tob Res* **4:** 149–159.

Tonetti MS (1998). Cigarette smoking and periodontal diseases: etiology and management of disease. *Ann Periodontol* **3:** 88–101.

Vine MF, Hulka BS, Margolin BH *et al*. (1993). Cotinine concentrations in semen, urine, and blood of smokers and nonsmokers. *Am J Publ Health* **83:** 1335–1338.

Warnakulasuriya S (2002). Effectiveness of tobacco counselling in the dental office. *J Dent Edu* **66:** 1079–1087.

Watt R, Daly B (2003). Prevention. Part 1: Smoking advice within general dental practice. *Brit Dent J* **194:** 665–668.