Some Properties of Saliva Cotinine Measurements in Indicating Exposure to Tobacco Smoking

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Abstract: The studies reported herein were designed to investigate some properties of saliva cotinine measurements in indicating exposure to tobacco smoke. Such measurements were found to be minimally affected by saliva flow rate or time since smoking as well as being sensitive to a low level of exposure to tobacco smoke. Results supported the view that the saliva cotinine assay is the most useful currently available method for objectively measuring exposure to tobacco smoke. (*Am J Public Health* 1986; 76:1245–1246.)

Introduction

Cotinine has several advantages over other biochemical measures of smoking; the only study to have compared smoking status classification error rates using blood cotinine, SCN (thiocyanate), and COHb (carboxyhemoglobin) measures achieved significantly lower error rates with cotinine.[1] Most previous work with cotinine has involved blood or urine sampling, often impractical procedures.[1–3] The experiments reported here were therefore designed to investigate some factors which may influence cotinine levels measured in saliva.

Methods

Experiment 1—Effects in Regular Smokers of Smoking One Cigarette and of Variation in Saliva Flow Rate on Cotinine Level

Subjects were 12 smokers who usually smoked a mean of 19.5 cigarettes per day and had smoked a mean of 5.8 cigarettes on the day of, but prior to, the experiment.

At around noon, approximately an hour after the most recent cigarette, each subject provided two samples of "mixed" saliva, 15 minutes apart. One sample was provided by rapid tongue and cheek movements (RTC), the other with salivary flow rate stimulated by a piece of orange candy (Lifesavers) in the mouth. Order of sample delivery method was counterbalanced. Samples were approximately 3 gm in weight, delivered as quickly as possible into a 6 ml plastic container and timed with a stopwatch.

Eight subjects then smoked a 1.0 mg nominal nicotine yield cigarette and then provided three more saliva samples (by candy method) 0, 30, and 120 minutes after finishing the cigarette. Samples were frozen until assayed.[4]

Experiment 2—Measurability of Saliva Cotinine Level after Just One Cigarette

Five nonsmokers and two usually light smokers (5, 10 cigarettes per day) who abstained from smoking for one week prior to the experiment, acted as subjects. Subjects smoked one cigarette (1 mg nominal, nicotine yield) at about 6 pm. They were asked to inhale the smoke fairly deeply, resulting in reports of mild post-smoking nausea from most. Saliva samples were provided (by RTC method) 2, 12, 24, and 48 hours after smoking. Subjects were asked to avoid smoking during this period, and, being known personally by the first author, were considered completely reliable in their self-reports of having done so.

Results

Experiment 1

Counterbalancing was effective, with no differences observed between first and second presmoking samples in mean flow rate (2.06 and 2.05 gms/min, respectively; n.s.) or mean cotinine level (1628, 1672 nMol/ml, respectively; n.s.).

However, the RTC and candy delivery methods resulted in significantly different mean flow rates (0.88, 3.23 gms/min, respectively; $t = 12.2$, $p < 0.001$) and mean cotinine levels (1807, 1493 nMol/ml, respectively; $t = 4.0$, $p < 0.01$).

For the eight subjects who provided four candy-facilitated saliva samples, mean cotinine levels were, in sequential order, 1414, 1534, 1494 and 1556 nMol/ml, respectively.

Three orthogonal contrasts compared the presmoking cotinine level with the mean of the post-smoking levels and tested for linear and quadratic trends in the post-smoking levels. None of these contrasts detected trends (t's = 1.08, 0.26, 0.71, respectively).

For comparison, nicotine levels were measured in samples from four of these eight subjects and found to be 3777, 46989, 6253 and 3219 nMol/ml, respectively.

Finally, to estimate assay reliability, cotinine levels in each of the four candy-facilitated samples were correlated, pairwise, with each other. Correlations ranged from 0.93 to 0.97.

Experiment 2

Mean saliva cotinine levels 2, 12, 24, and 48 hours after previously abstinent subjects smoked one cigarette were 77, 70, 30, and 10 nMol, respectively. A "blank" sample of distilled water analyzed at the same time was found to have 11 nMol/l of cotinine in it.* Thus it was possible to distinguish 7/7 two and 12-hour samples, 5/7 24-hour samples, and 3/7 48-hour samples from the blank. Cotinine levels in samples collected in the evening (2, 24, and 48 hour samples) indicated a half-life of about 16 hours.

Discussion

All objective measures of exposure to tobacco smoke have characteristics which limit their application. For example, recent smoking can markedly affect measured levels of carbon monoxide (CO),[6,7] nicotine,[8] and thiocyanate (SCN) in saliva.[9] In contrast, saliva cotinine level was found in the present study to be minimally affected by smoking immediately prior to saliva sample collection.

Moreover, cotinine's half-life of 16–25 hours[2,10] is longer than those of nicotine[4,11] and carbon monoxide,[12] thus reducing the need to control, or correct assay results for, time of day of sample collection.

In addition, saliva cotinine levels' relative independence of salivary flow rate contrast with the effect of flow rates on urinary or saliva nicotine levels[13,14] or saliva thiocyanate

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*The assay laboratory was not smoke-free.[5]

TABLE 1—Results of Experiment 2

Hours after Smoking	Cotinine levels (nMol/l)							
	Subject Number							
	1	2	3	4	5	6	7	$\bar{X}$
2	80	83	80	87	45	37	126	77
12	73	58	67	93	33	18	147	70
24	24	27	49	59	0	11	43	30
48	10	9	20	14	0	2	16	10

level.[15,16] In experiment 1, the increase in saliva flow rate by a factor of 3.7 produced by the candy was associated with an apparent reduction in mean cotinine concentration to 83 per cent of the mean RTC level. A pilot study, involving only a single subject and several methods of increasing saliva flow rate, suggested that this small reduction may in fact have been due to candy-facilitated samples having been partly constituted of dissolved sugar rather than saliva.

Finally, nonsmokers have variable, non-zero levels of both CO and SCN, making it difficult to discriminate them from low-rate smokers with these measures. For example, saliva SCN levels have been found to identify only 12 per cent of adolescents[17] and 43 per cent of adults[18] who reported smoking one to five cigarettes per day.

The essentially zero cotinine levels found in nonsmokers, coupled with cotinine's demonstrated high sensitivity to inhalation of tobacco smoke, indicate that cotinine measurement will generally provide a less ambiguous indication of regular low-rate smoking than will SCN or CO measurement.

In fact, most people who inhale the smoke from at least two or three cigarettes per day, as well as higher-rate smokers sampled even after some days of abstinence, should be distinguishable from nonsmokers by saliva cotinine assays, assuming that a smoke-free assay laboratory is used.[5] Such low-rate smokers may, however, be difficult to distinguish from nonsmokers who are exposed to a significant degree of passive smoking.[19]

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