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Cotinine as a biomarker of systemic nicotine exposure in spit tobacco users

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Abstract

Unlike cigarette smokers, spit tobacco (ST) users absorb a significant amount of nicotine through the gastrointestinal tract while swallowing tobacco juice. The majority of the absorbed nicotine is rapidly converted to cotinine during first-pass hepatic metabolism. This process potentially compromises the utility of cotinine as a biomarker for systemic nicotine exposure in ST users. To investigate this question, we correlated nicotine and cotinine concentrations with clinical measures of ST use in 68 daily ST users enrolled in a nonnicotine pharmacologic intervention trial. We found that a higher frequency of swallowing tobacco juice (P=.007) was an independent predictor of higher serum cotinine concentrations. Serum nicotine concentrations, on the other hand, were not correlated with a higher frequency of swallowing. In the absence of a reliable way to measure frequency of swallowing, we conclude that cotinine should not be used for guiding clinical decisions that depend upon a precise quantification of systemic nicotine exposure, such as tailored nicotine replacement therapy.

Keywords: Nicotine; Cotinine; Smokeless tobacco; Chewing tobacco; Biomarker

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1. Introduction

Cotinine is the major metabolite of nicotine and a specific biomarker for nicotine exposure in cigarette smokers (Benowitz & Jacob, 1984). Serum cotinine concentrations have been shown to be associated with relapse to smoking and to correlate with the number of cigarettes smoked per day, the degree of nicotine dependence, and the severity of nicotine withdrawal symptoms following smoking cessation (Hall, Herning, Jones, Benowitz, & Jacob, 1984; Pomerleau, Fertig, & Shanahan, 1983; U.S. Department of Health and Human Services, 1988).

In contrast to smokers who absorb nicotine primarily through the pulmonary vasculature, spit tobacco (ST) users absorb nicotine both through the buccal mucosa and the gastrointestinal tract (Benowitz, Jacob, & Yu, 1989). The swallowed nicotine is known to enter the portal circulation through the small intestine and undergo first-pass metabolism by the liver. The nicotine entering the liver through the portal circulation is converted to cotinine and other metabolites before reaching the systemic circulation, contributing to the higher serum cotinine concentrations observed in ST users compared with cigarette smokers (Benowitz et al., 1989). Cotinine may be an inaccurate measure of systemic nicotine exposure in ST users if it varies with the amount of tobacco juice that is swallowed.

In this study, we assessed the correlations between serum nicotine and cotinine concentrations with clinical measures of ST use using data obtained from 68 daily ST users enrolled in a nonnicotine pharmacologic intervention trial.

2. Methods

We analyzed the baseline serum concentrations of nicotine and cotinine from 68 adult (age ≥ 18 years old) regular users of ST enrolled in a prospective, randomized, placebo-controlled clinical trial. This trial was designed to assess the efficacy of bupropion SR 300 mg/day taken for 12 weeks in conjunction with behavioral intervention for increasing ST abstinence rates. Details of the study design are reported elsewhere (Dale et al., 2002).

Nicotine dependence was measured using a nine-item questionnaire based upon the Fagerström Tolerance Questionnaire modified and tested for use with ST users (Boyle, Jensen, Hatsukami, & Severson, 1995). A strong correlation between the scale and salivary cotinine concentrations ($n = 121$) has been shown [$r = 0.33$; $P < .01$] (Boyle et al., 1995).

At the baseline visit, blood was collected in the afternoon between 30 and 60 min after placement of the usual ST dose during ad libitum use (Benowitz et al., 1989; Benowitz, Porchet, Sheiner, & Jacob, 1988).

2.1. Nicotine and cotinine measurements

These tests were performed by liquid chromatography/triple quadrapole mass spectrometry (LC/MS/MS) (Moyer et al., 2002).

Table 1
Baseline serum concentrations of nicotine and cotinine

	n	Median	Mean ± S.D.	Range
Nicotine, ng/ml				
Overall	64	22	23 ± 11	7–48
Chewing tobacco only	26	22	23 ± 10	9–42
Snuff only	36	23	24 ± 11	7–48
Cotinine, ng/ml				
Overall	68	402	454 ± 256	102–1230
Chewing tobacco only	27	397	450 ± 244	102–1183
Snuff only	39	429	471 ± 265	167–1230

Of 68 subjects, there were 39 who used snuff only, 27 who used chewing tobacco only and 2 who used both snuff and chewing tobacco. Cotinine and nicotine concentrations were not significantly different for subjects that used snuff only versus chewing tobacco only (P=.803 for nicotine, P=.860 for cotinine). For the two subjects that reported using both snuff and chewing tobacco, the nicotine concentrations were 6 and 13 ng/ml and cotinine concentrations were 125 and 236 ng/ml.

2.2. Statistical analysis

The Spearman rank correlation was used to assess the univariate association of tobacco use variables with serum nicotine and cotinine concentrations. The tobacco use variables considered in this analysis included the average number of cans/pouches used per week, the average number of dips per day, the length of time a single dip is kept in the mouth, the time since last dip, and the frequency of tobacco juice swallowing (0 = never, 1 = sometimes,

Table 2
Association of tobacco use variables with serum nicotine and cotinine concentrations[a]

Characteristic	Mean ± S.D.	Nicotine		Cotinine	
	Median (Range) ng/ml	r[a]	P	r[a]	P
Average dips/chews per day	12.8 ± 10.2 10 (3–70)	.27	.038	.35	.004
Average cans/pouches per week	3.3 ± 2.1 3.0 (1–11)	.39	.001	.42	< .001
Length of time one dip/chew is kept in mouth, minutes	82.9 ± 131.9 45 (10–930)	.22	.085	.15	.217
Time since last dip/chew, hours	0.9 ± 0.5 0.9 (0–2.2)	.15	.257	.13	.279
Swallow tobacco juice[b]	1.1 ± 0.8 1 (0–2)	.08	.517	.26	.032

[a] Univariate associations were evaluated using Spearman rank correlation. There are 68 subjects included, 4 subjects were missing data for serum nicotine, 3 subjects were missing average dips per day and 1 subject was missing time since last dip.

[b] Frequency of swallowing tobacco juice was quantified as 0 = never (n = 20), 1 = sometimes (n = 22), and 2 = always (n = 26).

2 = always). The rank sum test was used to compare tobacco use variables and serum nicotine and cotinine concentrations for subjects that used snuff only versus chewing tobacco only. Multiple linear regression was employed to determine whether the frequency of swallowing tobacco juice was associated with serum cotinine concentration after adjusting for the average number of cans/pouches used per week. Serum cotinine data were transformed using a logarithmic transformation for the multivariate analysis. In all cases, two-sided tests were used with P values ≤ 0.05 considered statistically significant.

3. Results

These analyses are based on baseline laboratory data from 68 (67 males, 1 female) subjects. The mean ($\pm$ S.D.) age of the subjects was 36.5 $\pm$ 12.5 years (range, 24–79 years) with an average number of years of regular ST use of 17.1 $\pm$ 11.3 years (range, 3–63 years). Thirty-nine of the 68 subjects (57%) used only snuff [moist ground tobacco], 27 (40%) used only chewing tobacco [cut tobacco leaves], and 2 (3%) used both snuff and chewing tobacco.

Concentrations of serum nicotine and cotinine did not differ significantly between subjects who used only snuff or only chewing tobacco (Table 1). Subjects who used only snuff were

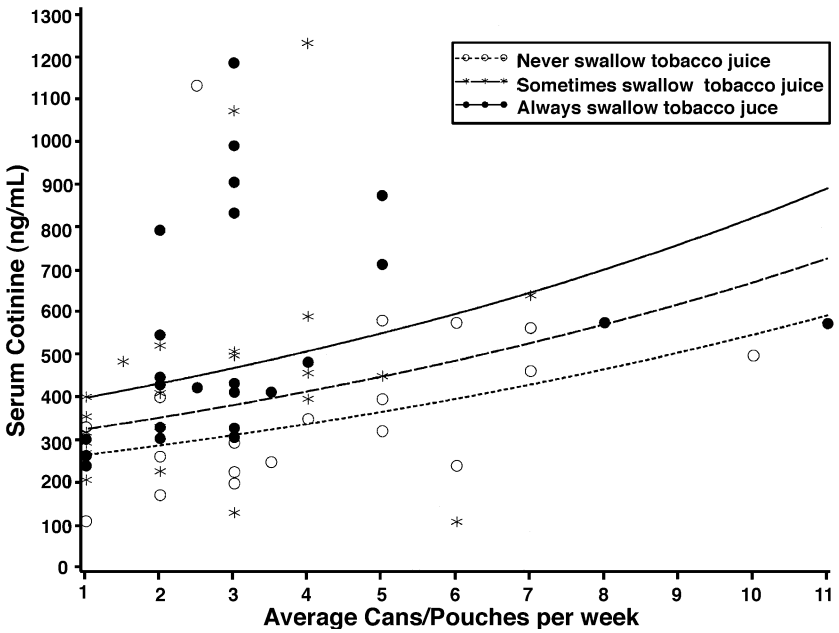

Fig. 1. The frequency of swallowing tobacco juice was quantified as 0 for never ($n = 20$), 1 for sometimes ($n = 22$), and 2 for always ($n = 26$). From multivariate analysis, higher serum cotinine concentration was associated with higher cans/pouches per week (P=.007) and higher frequency of swallowing tobacco juice (P=.007). Reference lines correspond to the fitted regression: $\log_{10}$ (serum cotinine) = 2.38 + 0.090 × Swallowing Frequency + 0.035 × Cans or Pouches per Week.

similar to those who used only chewing tobacco with respect to the average dips/chews per day (median 10 dips vs. 10 chews for snuff and chewing tobacco respectively; P=.916), the average number of cans/pouches per week (median 3 cans vs. 3 pouches; P=.853), the average time one dip/chew was left in the mouth (median 45 vs. 60 min; P=.488), and the time since the last dip (median 0.8 vs. 0.9 h; $P = 0.836$). However, there was evidence to suggest that the frequency of swallowing tobacco juice was higher in those that used snuff only (15% never, 37% sometimes, 48% always) compared with those that used chewing tobacco only (41% never, 28% sometimes, 31% always) (P=.039).

A higher number of cans/pouches used per week (Spearman $r = 0.42$; $P < .001$), a higher number of dips per day ($r = 0.35$; P=.004), and an increased frequency of swallowing tobacco juice ($r = 0.26$; P=.032) were univariately associated with a higher serum cotinine concentration. A higher number of dips per day ($r = 0.27$; P=.038) and a higher number of cans/pouches used per week ($r = 0.39$; P=.001) were univariately associated with higher serum nicotine concentration (Table 2). Using multiple linear regression, an analysis was performed to determine whether the frequency of swallowing tobacco juice was independently associated with serum cotinine concentration after adjusting for the amount of tobacco used, quantified as the number of cans/pouches per week. From this analysis, a higher frequency of swallowing tobacco juice (P=.007) and higher cans/pouches per week (P=.007) were found to be independent predictors of higher serum cotinine concentration (Fig. 1).

4. Discussion

In this study, we found that higher frequencies of tobacco juice swallowing are associated with higher serum cotinine concentrations but not with higher serum nicotine concentrations. This finding suggests that cotinine is not an accurate biomarker of systemic nicotine exposure in ST users, which has important implications for research and clinical practice.

The use of cotinine as a biomarker of systemic nicotine exposure for tobacco users originates from studies of cigarette smokers. Cotinine has been proposed as a valid surrogate biomarker of nicotine exposure in smokers since higher serum cotinine concentrations correlate with higher scores on nicotine dependence measures (Pomerleau, Pomerleua, Majchrzak, Kloska, & Malakuti, 1990). Cotinine has several advantages over the measurement of nicotine in the quantification of systemic nicotine exposure. First, cotinine is easier to assay than nicotine for patients in a clinical setting since it has a longer half-life (15–24 h vs. 2–3 hours, respectively) and is present in higher serum concentrations (Benowitz, 1988; Lawson et al., 1998). Secondly, the negligible gastrointestinal absorption of nicotine during cigarette smoking ensures that the measured serum cotinine is reflective of nicotine concentrations to which the central nervous system is exposed. These properties allow for the clinical use of cotinine for the tailoring of nicotine replacement therapy in cigarette smokers (Hays et al., 2001). The findings of the current study indicate that a different approach is needed for making decisions that rely on an accurate quantification of systemic

nicotine exposure in ST users, such as dose adjustments during nicotine replacement therapy. In order to tailor nicotine replacement therapy in ST users using serum cotinine concentrations as a guide, a precise determination of swallowing frequency would be necessary to avoid underdosing or overdosing. Even if such an assessment were to be developed, self-reported tobacco juice swallowing frequency may not be accurate.

In this study, we also found that snuff users and chewing tobacco users have similar nicotine and cotinine serum concentrations. The finding that ST users who use only snuff or only chewing tobacco are similar in their patterns of use and in their nicotine concentrations has research implications. ST researchers have traditionally randomized snuff only or chewing tobacco only subjects as if they had identical exposure to nicotine. In order for this to be valid, we have to assume that one can of snuff is equal to one pouch of chewing tobacco. Our finding that snuff users and chewing tobacco users have similar numbers of dips/chews per day, times per dip/chew, cans/pouches consumed per week, and similar concentrations of nicotine in the serum allows us to continue to operate under this assumption.

We also found that cotinine correlates significantly with traditional measures of nicotine dependence in ST research (i.e., average dips/chews per day and the number of cans/pouches used per week). Our findings support those of a previously published study (Boyle et al., 1995). Similar to the previous authors, we found that the question "How often do you swallow your tobacco juice rather than spit (always, sometimes, never)" on the nicotine dependence questionnaire was positively and significantly correlated with cotinine concentrations. Both cotinine concentrations and swallowing frequency appear to correlate with the degree of nicotine dependence. However, if we use cotinine to guide nicotine replacement therapy in ST users, we may overdose ST users who are less dependent but swallow more and underdose more dependent ST users who never swallow.

The major methodologic difference between our study and the previous study is that we used serum cotinine concentrations for the analyses rather than salivary cotinine (Boyle et al., 1995). Although salivary cotinine concentrations are 20–40% higher than serum cotinine concentrations, salivary and serum cotinine have similar half-lives and are highly correlated (Curvall, Elwin, Kazemi-Vala, Warholm, & Enzell, 1990). Therefore, if cotinine does not correlate with systemic nicotine exposure in the serum it will not, theoretically, correlate with it in the saliva.

One limitation of this study relates to the performance of multiple comparisons with measured serum nicotine and cotinine concentrations. However, our results are similar to those of other authors which decreases the likelihood that our findings are statistically significant through random chance (Boyle et al., 1995).

We conclude that cotinine should not be used for guiding clinical decisions that depend upon a precise quantitation of systemic nicotine exposure, such as adjustments in nicotine replacement therapy. While cotinine may not accurately reflect systemic nicotine exposure, it may be useful for differentiating ST users from those who have stopped using tobacco. We propose that serum nicotine concentration is a more reliable way to assess systemic nicotine exposure in ST users and should serve as the biomarker for guiding nicotine replacement in this population of tobacco users.

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