Nicotine & Tobacco Research Volume 7, Number 3 (June 2005) 469–474

Brief Report

Smokeless tobacco topography and toxin exposure

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[Received 20 November 2003; accepted 16 March 2005]

This study examined the relationship between oral smokeless tobacco (ST) topographical measures and biomarkers of tobacco and carcinogen exposure. During 2 weeks of baseline ad libitum ST use, 54 male participants (mean age = 32.1 ± 7.5 years) were required to record the time each dip was placed in and removed from their mouths. Urine samples were collected twice during baseline and analyzed for nicotine, cotinine, and their glucuronides (total nicotine and total cotinine) and for metabolites of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides (total NNAL). The results suggest that frequency and duration measures of ST use ($p<.01$ to $p<.001$), particularly total dip duration, are significantly correlated with total cotinine, total nicotine, and total NNAL. These results suggest that how long a ST user has tobacco in his mouth is most predictive of tobacco toxin exposure.

Introduction

An estimated 6.7% of U.S. high school students have used oral smokeless tobacco (ST) within the past month, and male students are more likely to use ST than are female students (Grunbaum et al., 2003). The use of ST by adolescents is a concern, especially if they continue to use ST into adulthood. In 2001, approximately 7.3 million (3.2%) noninstitutionalized individuals aged 12 years or older were using ST in the United States (Substance Abuse and Mental Health Services Administration, 2002). Chronic ST use can result in nicotine addiction as well as several adverse health effects including periodontal disease, cancer (oral cavity and pancreas), and increases in cardiovascular disease risk factors (Hatsukami, Lemmonds, & Tomar, 2004; International Agency for Research on Cancer, 1985; U.S. Department of Health and Human Services, 1986; Vainio & Weiderpass, 2003).

The uptake of several ST toxins is associated with these negative health effects. Nicotine leads to addiction, activates the sympathetic nervous system, and can result in fetal toxicity (Benowitz, 1998). Tobacco-specific *N*-nitrosamines (TSNAs) are one of the most potent classes of carcinogens in ST. TSNA levels vary across different brands of ST. The most popular products (Copenhagen and Kodiak) that have the largest market share contain the highest levels of nicotine and TSNAs, whereas starter products such as Skoal Bandits contain lower levels of nicotine and carcinogens (Hoffmann et al., 1995). The two main carcinogenic TSNAs in ST are N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK; Hecht & Hoffmann, 1988, 1989; Hoffmann et al., 1995). A mixture of NNK and NNN causes oral and lung tumors in rats, and these carcinogens are believed to be involved in the formation of oral cancer in humans (Hecht, 1998).

No studies have analyzed NNN or its metabolites in human subjects; however, more is known about the carcinogen NNK. NNK is metabolized to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL),

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ISSN 1462-2203 print/ISSN 1469-994X online
DOI: 10.1080/14622200500135640

which is also carcinogenic. NNAL is further metabolized by glucuronidation or oxidation (for review, see Hecht, 1998). The urinary metabolites NNAL and its glucuronides are accepted biomarkers of NNK exposure (Hecht, 2002).

The extent of exposure to toxins in ST depends on a number of factors including the levels of toxins within the product, the pH of the product, the way an individual uses the product, and individual differences in uptake and metabolism of toxins. Some of the factors related to the use of the product include quantity, frequency, and duration of ST use. The present study examined the relationship between levels of nicotine, cotinine, and their glucuronides (markers of tobacco exposure) and NNAL plus its glucuronides (markers of carcinogen exposure) with self-reported measures of tobacco use such as ST tins used per week, total dips per day, total daily dip duration, and total daily dipping time (time from first dip in the morning until last dip of the day). This information would be valuable in determining the aspects of ST use that are most associated with the extent of tobacco toxin exposure. In addition, the results may provide information necessary to develop methods by which reduction in tobacco toxin exposure can be achieved among those interested in reducing but not quitting tobacco use (Hatsukami et al., 2003).

Method

Subjects

Male ST users aged 21–65 years were recruited from the local metropolitan area for a study at the University of Minnesota comparing nicotine replacement products and new tobacco products. To be eligible to participate, subjects had to be using at least one tin of ST per week for a minimum of 1 year. Subjects were excluded if they had unstable medical conditions or were using other tobacco products or any methods for quitting or cutting down tobacco use.

Measures

Self-reported tobacco use information was elicited from each subject. Subjects were required to keep daily paper diaries of their tobacco use. In each diary, subjects were required to write down when they put a dip into their mouth, when they removed a dip from their mouth, and when a new tin of ST was opened.

Urine samples, the first void in the morning, were collected twice during baseline ad libitum use. These samples were analyzed for nicotine plus its glucuronides (total nicotine) and cotinine plus its glucuronides (total cotinine) as measures of tobacco exposure using gas chromatography–mass spectrometry (Hecht et al., 1999). The samples were also analyzed for NNAL plus its glucuronides (total NNAL) as measures of carcinogen exposure using GC-TEA (S. Carmella, S. Han, A. Fristad, Y. Yang, & S. Hecht, 2003).

Statistical analysis

The data collected from each subject included demographics, tobacco use history, total dips of ST per day, tins per week, average total daily dip duration, average daily dip duration of an individual dip, and total daily dipping time. The average total daily dip duration was calculated by adding the total daily dip duration (the total time that any dip was in the mouth) for a day of the baseline and then dificing by the total number of days in the base period. The average daily dip duration of an individual dip was calculated by first dividing the total number of minutes that a subject dipped for an entire day by the total number of dips for the day, and then summing up the resultant values over all the days during the baseline period, or finally dividing the sum by the total number of days during that period. The two baseline urine measurements for total nicotine, total cotinine, total nicotine plus total cotinine, and total NNAL were averaged to provide a mean value for each subject.

The correlations between continuous variables were evaluated using Spearman's rank correlation coefficients because most of the variables did not have a normal distribution. Some transformations were performed to improve the normality of distributions and equality of variances. The outcome variables total nicotine plus total cotinine and total NNAL were transformed into a square root scale, total cotinine into a natural log scale, and total nicotine was not transformed. The predictor variables, number of quit attempts, tins per week, dips per day, and average daily dip duration of an individual dip were transformed into a natural log scale, whereas others were not transformed.

The linear regression analysis was conducted using the transformed data to determine the associations between self-reported measures of ST use and each of the biomarker outcomes—total nicotine, total cotinine, total nicotine plus total cotinine, and total NNAL levels. A stepwise procedure was used to select the final linear regression model that contained the most significant predictors for the outcomes. Model assumptions, multicollinearity, and cases with outliers were checked using diagnostic tools. A test result with a p value of less than .05 was statistically significant.

Results

Study participants were 54 male ST users (52 Whites and 2 Asians). Their average age was 32.1 years ($SD=7.5$, range=21–58 years). Tobacco use and carcinogen biomarkers are described in Table 1.

Correlation analysis

Correlation values are displayed in Table 2. Average total daily dip duration, average daily dip duration of an individual dip, and total daily dipping time were significantly correlated with all four biomarkers. Figure 1 shows the scatterplot between total daily dip duration and total NNAL. Dips per day was significantly correlated with total cotinine, total nicotine plus total cotinine, and total NNAL. Tins per week was correlated with only total nicotine and not any of the other biomarkers. Other significant correlations included age with total nicotine, total cotinine, and total nicotine plus total cotinine; quit attempts with total NNAL; and years of ST use with total cotinine and with total nicotine plus total cotinine. Total nicotine, total cotinine, total nicotine plus total cotinine, and total NNAL were significantly correlated with each other. Figure 2 shows the scatterplot between total cotinine and total NNAL levels.

Regression analysis

The most significant predictors of outcome for the four biomarkers were determined using a stepwise selection. For total cotinine, total nicotine plus total cotinine, and total NNAL, only average total daily

Table 1. Tobacco use and carcinogen exposure.

Variable	*N*	Median	Mean ± standard deviation
Quit attempts	52	4.0	7.8 ± 14.1
Tobacco brand			
Copenhagen, fine cut	15		
Copenhagen, long cut	4		
Kodiak, fine cut	5		
Kodiak, long cut	20		
Skoal, long cut	7		
Other	3		
Years of smokeless tobacco use	54	12.0	12.5 ± 5.8
Dips/day	54	6.1	6.8 ± 3.0
Tins/week	53	2.8	3.4 ± 2.8
Average total daily dip duration, minutes[a]	53	388.0	423.0 ± 224.4
Average daily dip duration of an individual dip, minutes[b]	53	61.6	71.2 ± 44.3
Total daily dipping time, minutes[c]	54	860.0	820.6 ± 120.4
Total nicotine, nmol/mg creatinine	54	9.9	10.5 ± 5.6
Total cotinine, nmol/mg creatinine	54	20.1	23.5 ± 14.3
Total nicotine+total cotinine, nmol/mg creatinine	54	30.6	34.0 ± 18.3
Total NNAL, pmol/mg creatinine	54	2.9	3.3 ± 2.1

Notes. NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides.
[a]Total number of minutes per day that dip was in the mouth.
[b]Average daily number of minutes that an individual dip was in the mouth.
[c]Time from first dip in the morning until last dip of the day.

Table 2. Spearman's rank correlation coefficients.

Variable	Total nicotine	Total cotinine	Total nicotine+total cotinine	Total NNAL
Age	0.37**	0.40**	0.42**	0.21
Quit attempts	−0.24	−0.21	−0.22	−0.31*
Years of smokeless tobacco use	0.21	0.27*	0.27*	0.12
Dips/day	0.19	0.39**	0.35**	0.29*
Tins/week	0.28*	0.16	0.19	0.11
Average total daily dip duration[a]	0.50***	0.60***	0.58***	0.64***
Average daily dip duration of an individual dip[b]	0.37**	0.35*	0.34*	0.44**
Total daily dipping time[c]	0.57***	0.59***	0.60***	0.50***
Total nicotine	—	0.72***	0.83***	0.37**
Total cotinine	—	—	0.98***	0.63***
Total nicotine+total cotinine	—	—	—	0.58***

Notes. NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides.
[a]Total number of minutes per day that dip was in the mouth.
[b]Average daily number of minutes that an individual dip was in the mouth.
[c]Time from first dip in the morning until last dip of the day.
*$p<.05$; **$p<.01$; ***$p<.001$.

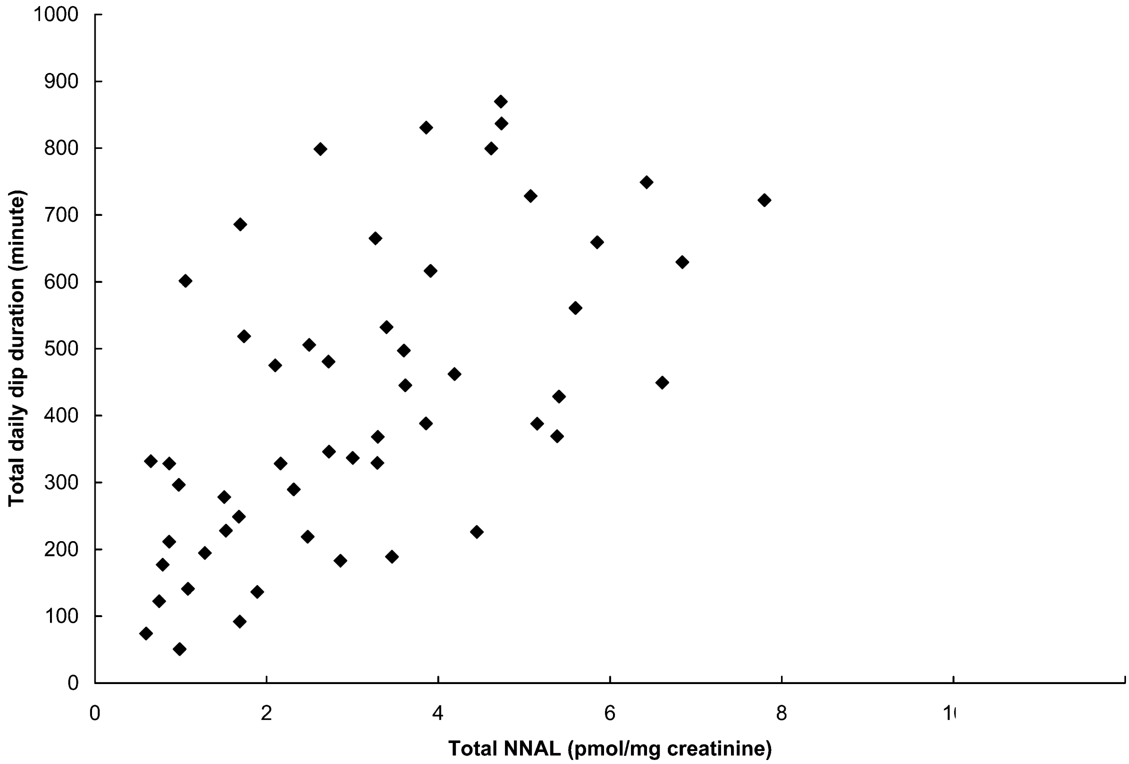

Figure 1. Scatterplot of total daily dip duration against total NNAL level.

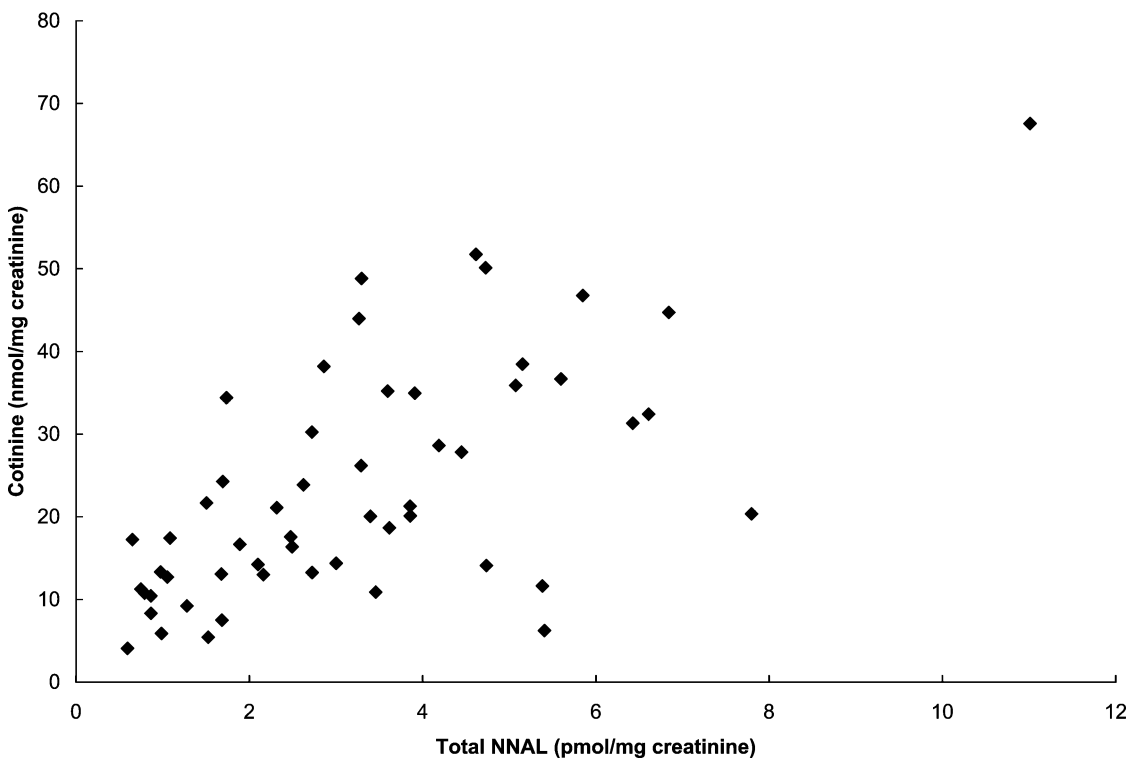

Figure 2. Scatterplot of total cotinine level against total NNAL level.

dip duration remained in the final model. Average total daily dip duration was significantly and positively correlated with total cotinine (t=5.27, p<.0001), total nicotine plus total cotinine (t=5.30, p<.0001), and total NNAL (t=5.54, p<.0001). As the average total daily dipping duration increased by 1 min, total cotinine increased by 0.002 unit (nmol/mg creatinine in natural log scale), total nicotine plus total cotinine by 0.004 unit (nmol/mg creatinine in a square root scale), and total NNAL by 0.001 unit (pmol/mg creatinine in a square root scale).

For total nicotine, not only average total daily dip duration but also age and tins per week remained in the final model. After adjusting for the other two covariates, age was significantly related to total nicotine (t=2.14; p=.0377); a 1-year increase in age was associated with a 0.240 nmol/mg creatinine increase in total nicotine. Tins per week was also significantly associated with total nicotine (t=2.38, p=.0214); a one-unit increase in tins in a natural log scale was associated with a 2.72 nmol/mg creatinine increase in total nicotine. Finally, average daily total dip duration was significantly related to total nicotine after covariate adjustment (t=3.07, p<.0035); a 1-min increase was associated with an increase of 0.01 nmol/mg creatinine of total nicotine.

Discussion

The data suggest that frequency and duration of ST use but not necessarily the amount of ST use are strongly correlated with biomarkers of tobacco and carcinogen exposure. Most previous research has focused on the correlation between self-reported measures of ST use and salivary cotinine as the marker of tobacco exposure. One study found a significant correlation between salivary cotinine levels with four measures of tobacco exposure: total number of dips per day, minutes per dip, days per can, and weight of a dip (Severson, Eakin, Lichtenstein, & Stevens, 1990). Other studies have shown a significant correlation between cotinine levels and frequency and duration of ST use but no significant correlation between cotinine and weight or amount of ST dip or chew (Hatsukami, Anton, Callies, & Keenan, 1991; Hatsukami, Gust, & Keenan, 1987; Hatsukami, Keenan, & Anton, 1988). The result from the study showing a relationship between amount (weight per dip) and cotinine levels may be limited because the weight of only one "typical" dip was examined (Severson et al., 1990), unlike the other study that examined the weight of dips across days (Hatsukami et al., 1988). A limitation of the Severson et al., study was that no data were collected on grams of smokeless tobacco used per week.

One other study examined the correlation between ST use and carcinogen uptake. Hecht et al. (2002) showed a significant correlation between tins per week and total NNAL, unlike the present study, which found no such relationship. The Hecht et al. study had a small sample size (N=13) and did not examine other parameters of ST use. These equivocal findings on this measure would indicate that amount of tobacco use results in a weak correlation with uptake of NNK.

In the present study, the average duration of ST exposure per day was approximately 7 hr. This is a much longer duration of exposure than the 4.2 hr or 4.7 hr previously described in Hatsukami et al. (1988, 1991), which included younger ST users with shorter duration of use. This length of carcinogen exposure is a great concern because the present study found a significant correlation between duration of ST exposure and total NNAL levels. Kresty et al. (1996) observed a strong association between leukoplakia and increasing levels of NNAL and NNAL-Gluc, [4-(methylnitrosamino)-1-(3-pyridal)but-1-yl]-beta-O-D-glucosiduronic acid, indicating that higher levels of exposure are potentially associated with precancerous oral lesions.

Reducing tobacco toxin exposure among ST users may be an important goal for those who are unable or unwilling to quit. This reduction can be achieved either by switching to a product with lower carcinogen levels or by reducing the extent of tobacco use. Because frequency and duration of ST use were found to be significantly correlated with carcinogen exposure, this result would suggest that ST users who want to reduce exposure while using their normal brand of ST should consider not only the amount of ST but, more important, the duration that ST is kept in the mouth and the frequency of product use. To date, however, the only proven method for reducing health problems associated with ST use is no use at all.

Acknowledgments

This research was supported by NIH grants DA13333, DA07239, and DA14404. The first author currently works for GlaxoSmithKline.

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