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Brief report

Nicotine Metabolite Ratio Is Associated With Lozenge Use But Not Quitting in Smokeless Tobacco Users

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Abstract

Introduction: The nicotine metabolite ratio (NMR) of 3'-hydroxycotinine to cotinine is a noninvasive marker of the rate of nicotine metabolism. Fast metabolism (ie, a high NMR) is associated with lower cigarette smoking abstinence rates using transdermal nicotine replacement. We evaluated whether the NMR can be used to predict self-reported nicotine lozenge use and tobacco abstinence among smokeless tobacco users treated for tobacco dependence.

Methods: This was a secondary analysis of data from one arm of a large trial. Participants received quitting support materials and 4-mg nicotine lozenges by mail plus three coaching phone calls. Saliva kits were mailed for collection of saliva samples, which were analyzed for cotinine and 3'-hydroxycotinine. Self-reported tobacco and lozenge use were assessed at 3 months. Analyses were performed using Spearman rank correlation and logistic regression.

Results: Of the 160 saliva collection kits mailed, 152 were returned. The NMR was not significantly correlated with the baseline amount of smokeless tobacco used, the number of years of tobacco use, or the level of tobacco dependence as measured by the Severson Smokeless Tobacco Dependency Scale. The NMR was positively correlated with lozenge use ($r = 0.21$, $P = .015$), but it did not predict self-reported 7-day point prevalence abstinence at 3 months.

Conclusions: Fast metabolizers may need to self-administer more nicotine replacement in the form of nicotine lozenges to achieve the same clinical response achieved by slower metabolizers using fewer lozenges.

Introduction

Nicotine is metabolized to cotinine (COT) and subsequently to 3'-hydroxycotinine (3HC) by the hepatic enzyme CYP2A6.^{1,2} COT and 3HC can be measured in saliva, plasma, or urine.^{3,4} The ratio of 3HC to COT is a noninvasive marker of the rate of nicotine metabolism; it is highly correlated with the oral clearance of nicotine, and is a marker of CYP2A6 enzyme activity.⁴ The 3HC/COT ratio is referred to as the nicotine metabolite ratio (NMR), with a higher NMR corresponding to faster nicotine clearance.

Among cigarette smokers, the NMR is independent of time since the last cigarette and is reproducible.⁴⁻⁶ Using the NMR, smokers can be characterized as fast or slow metabolizers of nicotine.⁷ Fast metabolizers smoke on average more cigarettes per day⁸ and have greater puff volume and higher carcinogen exposure.⁹ Fast metabolism has been associated with reduced smoking abstinence rates when standard transdermal nicotine replacement therapy is used.^{10,11} A higher dose of nicotine replacement may increase smoking abstinence rates among fast nicotine metabolizers.⁷

Smokeless tobacco (ST) is tobacco consumed orally and not burned. The use of ST is the greatest exogenous source of human exposure to carcinogenic nitrosamines¹² and has been associated with oral and extra-oral cancers,^{13–16} as well as cardiovascular and cerebrovascular diseases.¹⁴ Few pharmacologic interventions have been shown to increase long-term ST abstinence rates (≥ 6 months).¹⁷ The use of clinical data and biomarkers may improve treatment and treatment dosing for ST users.

The NMR has been evaluated among ST users in the Alaska Native population by measuring 3HC and COT in plasma and urine.¹⁸ We sought to evaluate the utility of the NMR to predict nicotine lozenge use and tobacco abstinence in a population of ST users enrolled in a large randomized trial.

Methods

Study Overview

The current investigation was a secondary analysis of data from a large trial randomizing ST users to one of three conditions: (1) Assisted Self-Help, in which subjects received quitting support materials by mail plus three coaching phone calls; (2) Lozenge-Assisted Self-Help, in which subjects received quitting support materials and nicotine lozenges by mail plus three coaching phone calls; or (3) Lozenge Self-Help, in which subjects received quitting support materials and nicotine lozenges by mail, but no coaching phone calls.¹⁹ Prior to participant enrollment, the study was approved by Oregon Research Institute's Human Subjects Institutional Review Board and registered with <http://ClinicalTrials.gov> (NCT01341938).

ST users were recruited through online marketing. Subjects were eligible for inclusion if they were at least 18 years of age, were using ST as a primary tobacco product and had been using it daily for at least 1 year, were willing and motivated to quit in the next month, and had an email address and a US mailing address.

Potential participants expressing an interest in the study received a consent form and a baseline questionnaire with a postage-paid reply envelope. Following receipt of the signed consent form and completed baseline assessment, participants were randomized and mailed quitting support materials for their assigned experimental condition. Participants assigned to the lozenge conditions received Nicorette 4-mg nicotine lozenges in the mail along with an instruction sheet on their use. Participants were instructed to use one lozenge every 1 to 2 hours for the first 6 weeks, one every 2 to 4 hours for weeks 7 through 9, and one every 4 to 8 hours for weeks 10–12. Additional lozenges could be obtained from the research program during the 12-week quitting period with a maximum of 12 boxes (81 lozenges/box) of lozenges per participant. For the current study, only the Lozenge-Assisted Self-Help group was assessed for the NMR and only a subset of the participants in this study arm was analyzed. Tobacco abstinence was by self-report and assessed at 3 and 6 months after randomization. Self-reported abstinence for a study with the demand characteristics of the current design has been suggested to be a valid approach.²⁰

Nicotine Metabolites

After giving informed consent, 160 consecutive subjects assigned to the Lozenge-Assisted Self-Help cohort were selected to receive a saliva collection kit, which was mailed to their home. Of the 160 kits mailed, 152 were returned. Subjects provided samples before treatment began. Participants were instructed to wash their mouths out prior to providing the saliva sample. Detailed instructions on sample

collection and mailing the sample to a study coordinating center were provided. Saliva samples were sent for analysis to determine concentrations of COT and 3HC using liquid chromatography with tandem mass spectrometry.⁴ Biochemical analyses were conducted at the University of California, San Francisco.

Measurement of Tobacco and Lozenge Use

The level of tobacco dependence at baseline was measured with the Severson Smokeless Tobacco Dependency Scale.²¹ Tobacco use outcomes were self-reported and measured at 3 and 6 months postenrollment. For the current analysis we used the 3-month time point with the primary outcome being self-reported 7-day point prevalence tobacco abstinence for both ST and cigarettes.²²

To assess lozenge use, at the 3-month follow-up subjects were asked, "On the days that you used the lozenges, how much of each day did you use them?" with possible responses being: "little of the day," "less than half the day," "more than half the day," or "most of the day."

Statistical Analyses

The NMR was calculated as the ratio 3HC/COT. With the NMR treated as a continuous variable, Spearman rank correlation was used to assess the association of the NMR with tobacco history variables reported at baseline and with the amount of lozenge use during the day reported at the 3-month follow-up. To further assess whether the NMR was associated with abstinence, a univariate logistic regression analysis was performed with a log-transformed NMR treated as a continuous variable. In addition to the univariate analysis, a multivariable logistic regression analysis was performed with age, baseline Severson Smokeless Tobacco Dependency Scale²¹ and baseline cans/pouches per week included as covariates. In all cases, two-tailed *P* values $\leq .05$ were considered statistically significant.

Results

Participants

Characteristics of the participants from whom baseline salivary samples were collected are summarized in Table 1.

Nicotine Metabolite Ratio

The median NMR (25th, 75th percentile) was 0.25 (0.18, 0.38). The NMR was not observed to be significantly correlated with the baseline amount of ST used (Spearman rank correlation $r = 0.07$, $P = .41$), the number of years of tobacco use ($r = 0.15$, $P = .06$), or the level of tobacco dependence, as measured by the Severson Smokeless Tobacco Dependency Scale²¹ ($r = 0.01$, $P = .86$). The NMR was found to be positively correlated with the amount of self-reported lozenge use during the day (Spearman rank correlation, $r = 0.21$, $P = .015$; Figure 1). We also observed some evidence suggesting that baseline COT levels were associated with self-reported lozenge use ($r = 0.17$; $P = .047$).

Of the 152 participants included in the current study, 93 (61.2%) met criteria for self-reported 7-day point prevalence abstinence at 3 months. From logistic regression analyses, no evidence was observed regarding an association between the NMR and self-reported abstinence (unadjusted OR = 1.16 per 1 *SD* increase; 95% CI = 0.83, 1.61; $P = .386$; adjusted OR = 1.14; 95% CI = 0.81, 1.59; $P = .461$).

Table 1. Baseline Demographics, Tobacco History, and Nicotine Metabolites (*N* = 152)

Variable	Value
Age, years	
Mean $\pm$ SD	36.5 $\pm$ 10.7
Range	18–65
Gender, <i>n</i> (%)	
Male	148 (97)
Female	4 (3)
Race/ethnicity, <i>n</i> (%) ^a	
White/non-Hispanic	144 (96)
Other	6 (4)
Average ST use, cans/pouches/tins per week	
Mean $\pm$ SD	5.6 $\pm$ 2.9
Range	1–15
Years of regular ST use	
Mean $\pm$ SD	15.0 $\pm$ 9.3
Range	1–40
Severson Smokeless Tobacco Dependency Scale	
Mean $\pm$ SD	11.6 $\pm$ 4.0
Range	1–19
Nicotine metabolites	
COT, ng/mL	
Mean $\pm$ SD	410 $\pm$ 288
Median	360
25th, 75th percentiles	200, 571
3HC, ng/mL	
Mean $\pm$ SD	120 $\pm$ 121
Median	84
25th, 75th percentiles	52, 139
3HC/COT ratio (NMR)	
Mean $\pm$ SD	0.30 $\pm$ 0.19
Median	0.25
25th, 75th percentiles	0.18, 0.38

3HC = 3'-hydroxycotinine; COT = cotinine; NMR = nicotine metabolite ratio; ST = smokeless tobacco.

^aData were missing for two individuals. The "Other" group includes American Indian/Alaska Native (*n* = 1), Asian (*n* = 1), black/African American (*n* = 3), and white Hispanic (*n* = 1).

Discussion

In the current study, we observed that the NMR was correlated with a greater amount of self-reported lozenge use during the day among ST users who wanted to quit. However, the NMR was not associated with baseline ST use rates, level of tobacco dependence, or self-reported tobacco abstinence at 3 months. Our finding that level of tobacco dependence did not differ between fast and slow metabolizers was consistent with previous reports that the NMR is not correlated with the nicotine dependence score as measured by the Fagerström Test for Nicotine Dependence.^{8,23}

Some of our findings contrast with previous research in smokers in which the NMR ratio was associated with tobacco abstinence aided by nicotine patch treatment, but our results are consistent with findings in smokers treated with nicotine nasal spray. In a study of 480 treatment-seeking smokers randomly assigned to 8 weeks of transdermal nicotine or nicotine nasal spray, pretreatment NMRs predicted the effectiveness of the nicotine patch for smoking cessation.¹¹ However, it did not predict cessation with the use of the nicotine nasal spray. The authors hypothesized that faster metabolizers had worse outcomes than slower metabolizers with a fixed 21-mg patch dose because they required higher blood nicotine concentrations than those produced by the fixed patch dose. However, the nicotine nasal spray provided the freedom to titrate the nicotine dose to achieve desired blood nicotine levels during smoking cessation and would not be expected to be correlated with the NMR.

We observed a median NMR of 0.25, which is lower than previously observed (median 0.35²⁴). Although these previous investigations were with cigarettes smokers and our sample was composed of ST users, we do not suspect this accounts for the difference. One possibility is that the COT, a minor tobacco alkaloid thought to arise through tobacco processing or bacterial oxidation rather than bioprocessing by the tobacco plant²⁵ and which is present in ST, reduced the ratio because of recency of use relative to the timing of the saliva sample collection by the participant. In the current study, written instructions with graphics were provided to the participants instructing them to rinse their mouth out before providing the sample, which should have reduced the potential impact of this effect on

Figure 1. Nicotine metabolite ratio by amount of time lozenges used during the day.

the results. We did not ask the subjects to record the timing of the last tobacco use prior to sample collection.

We also did not find a significant association between the NMR and self-reported ST use, in contrast to some previous studies that showed the NMR to be positively correlated with baseline cigarettes smoked per day in cigarette smokers.¹¹ Our findings are consistent with a study of the NMR and self-reported ST use in Alaska Natives.²⁶ However, that study did find a significant association between the NMR and tobacco consumption as assessed by urine total nicotine equivalents or urine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), indicating that self-reported ST use is not an ideal measure of daily nicotine intake. The discrepancy may be explained by differences in nicotine content and pH among the different ST products and the influence of different patterns of use on nicotine delivery from these products.²⁵

Our study was limited by a small sample size adversely affecting our power to detect differences between fast and slow metabolizers. Our study was also limited by the use of self-reported abstinence and lozenge use and the timing of the assessment at 3 months when reporting may be influenced by outcome. These limitations may have compromised our ability to detect true differences due to an invalid self-report. The generalizability of our findings may be limited because of the low likelihood that any tailoring strategy would improve the high tobacco abstinence rates we observed in our sample population. Furthermore, although we observed a significant association between the NMR and the amount of self-reported lozenge use during the day, the magnitude of this effect is small and the clinical utility may be limited.

In summary, the NMR derived from measurements in saliva did not predict cessation of self-reported ST use aided by nicotine lozenge treatment, but it was associated with a greater amount of self-reported lozenge use during the day. Fast metabolizers may need to consume more nicotine replacement products to achieve the same clinical response as slow metabolizers. If ST users trying to quit tobacco self-titrate nicotine replacement product intake in response to their rate of nicotine metabolism, the use of a biomarker such as the salivary NMR might provide guidance on the amount of nicotine replacement therapy to use.

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Declaration of Interests

JOE has received consulting fees from GlaxoSmithKline, however, GlaxoSmithKline had no role in the conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication. JOE has received research support from Pfizer, Orexigen, and JHP Pharmaceuticals outside of the current study. NLB has served as a consultant to Pfizer and GlaxoSmithKline related to smoking cessation medications and has been an expert witness in litigation against tobacco companies. The other authors declared no competing interests.

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