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Measuring nicotine intake among highly-dependent adolescent smokers: Comparability of saliva and plasma cotinine concentrations

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Abstract

Cotinine is the most common biomarker used to assess nicotine exposure and abstinence. It can be measured in various matrices including saliva, plasma, and urine. Previous research with adults has shown high correlations between saliva and plasma cotinine concentrations. However, the research has not examined this relationship in adolescents. Additionally, variability in saliva flow and metabolism across gender, ethnicity, and age may impact the relationship between saliva and plasma cotinine concentration. Our aim was to examine the relationship between saliva and plasma cotinine concentration in a group of nicotine-dependent adolescent smokers. Additionally, we examined these correlations across gender, ethnicity and age. The sample consisted of 66 adolescent smokers (age 15.1±1.3, 63.6% girls, 66.7% European American, CPD 18.3±8.5, FTND 7.1±1.3). Saliva and plasma specimens were collected before the treatment phase of a nicotine replacement therapy trial and analyzed. The relationship between saliva and plasma cotinine concentration was analyzed using Pearson's correlation coefficients. We performed a secondary analysis using multiple regressions to compare correlations across race, gender and age. Results indicated a positive correlation between saliva cotinine and plasma cotinine concentration ($r=0.84$, $p<0.001$). Differences in correlations across age were significant ($t=3.03$, $p<0.01$). Differences across ethnicity approached significance ($t=-1.93$, $p=0.058$). Future research should seek to further validate saliva-to-plasma cotinine concentration ratios in adolescents as well as characterize saliva-to-plasma concentration differences and their underlying mechanisms. Published by Elsevier Inc.

Keywords: Adolescents; Nicotine Dependence; Cotinine; Bioverification

1. Introduction

Tobacco use kills 438,000 Americans each year and is the most preventable cause of death in the United States (CDC, 2005). More than 80% of these smokers will have started smoking before the age of 18 (USDHHS, 1994). Beginning in 1997, the adolescent smoking trend began to decline (CDC, 2006); however, recent data show that still approximately 3900 adolescents between the ages of 12 and 17 try smoking everyday and more than 1500 become daily smokers (SAMHSA, 2005). According to the National Center for Health Statistics, by grade twelve, 29% of boys and 23% of girls are current smokers with about half of them smoking on a frequent basis (20 days or more in the last 30 days) (NCHS, 2005). Thus, it is important to test and implement prevention and treatment interventions that have the potential to mitigate tobacco dependence in adolescent populations.

Several studies have tested the efficacy of nicotine replacement therapies (NRT) (Hanson et al., 2003; Hurt et al., 2000; Moolchan et al., 2005) and behavioral therapy (Krishnan-Sarin et al., 2006; Stevens et al., 2005) in adolescent populations. In order to determine the success of such interventions, an efficient means of testing nicotine exposure and abstinence beyond subjective self reporting must be available. Self-reporting, particularly among adolescents, has been shown to be only moderately reliable (Dolcini et al., 2003); therefore, self-report combined with biological markers has a greater ability to accurately reflect smoking status and degree of tobacco exposure. Due to its relatively long half-life, cotinine concentration is often used as a marker of tobacco exposure and abstinence (Benowitz and Jacob, 2002).

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Cotinine, the primary metabolite of nicotine, can be assayed in various biological fluids including blood, saliva, urine, and semen (Bramer and Kallungal, 2003). It is estimated that an average of 70–80% of the nicotine absorbed by a smoker is metabolized to cotinine (Bramer and Kallungal, 2003). Cotinine testing provides a more accurate measure than exhaled air carbon monoxide, one of the most widely-used biomarkers in smoking research (Dolcini, et al., 2003; Patrick et al., 1994), and has proved to be advantageous in examining nicotine intake due to its ability to estimate tobacco smoke exposure over a longer time frame than CO monitoring.

Cotinine has most frequently been assayed in plasma samples. Plasma cotinine concentration is highly correlated with tobacco smoke exposure from both direct smoke inhalation via cigarette consumption and environmental exposure via secondhand smoke (Eskenazi et al., 1995; Godtfredsen et al., 2006; Simoni et al., 2006; St. Charles et al., 2006).

Despite the reliability of plasma samples, drawing blood often presents logistical problems in a research setting (Benowitz and Jacob, 1994). Taking blood specimens is invasive, can cause stress and discomfort in some participants, and is impractical or even unethical in some instances (Jarvis et al., 2003). This is particularly true in adolescent populations where parental consent, adolescent decision-making ability, confidentiality, and treatment issues are major barriers to clinical trials (Moolchan and Mermelstein, 2002). Therefore using the least invasive means of objectively assessing nicotine intake is advantageous in smoking cessation trials with adolescents.

The use of saliva provides an alternative. Salivary testing offers a cost-effective, convenient, non-invasive method for assessing cotinine levels that eliminates the discomfort of drawing blood (Jarvis et al., 2003). Beyond the logistical advantages, previous research in adult populations suggests that saliva and plasma cotinine levels correlate highly and cotinine has a similar terminal half-life in both matrices. Rose et al. (1993) found that in adult populations saliva and plasma cotinine were highly correlated ($r=0.94$). More recently Jarvis et al. (2003) found a correlation between saliva and plasma cotinine of $r=0.99$ with saliva cotinine concentrations 25% higher than plasma concentrations. This finding is consistent with the SRNT Subcommittee on Biochemical Verification (2002) that states saliva cotinine concentrations are on average 10 to 40% higher than plasma cotinine levels. This range in variability might be partially caused by the amount of salivary gland stimulation; cotinine concentrations are lower in stimulated samples compared to unstimulated samples (Schneider et al., 1997).

The benefits of saliva sampling for cotinine further increase the need for validation of this method in an adolescent population. The aim of the present study was to explore whether the relationship seen in previous research with adult populations could be extended to an adolescent population. We compared saliva cotinine concentration with plasma cotinine concentration in heavily dependent adolescent cigarette smokers. Further, previous research on salivary flow rate and nicotine metabolism has shown variability across gender, ethnicity, and age which might impact the relationship of saliva and plasma cotinine concentrations (Berlin et al., 2007; Johnstone et al., 2006; Moolchan et al., 2006; Soderling et al., 1993; Yeh et al., 1998). Thus, we conducted a secondary analysis to examine potential differences in gender, ethnicity, and age.

2. Methods

2.1. Participants

Adolescent tobacco smokers were recruited through television, radio, and print advertisements as part of a three-arm, randomized, double-blind, double-dummy, placebo-controlled smoking cessation trial examining the safety, efficacy, and tolerability of two forms of NRT, nicotine patch and nicotine gum. The study was approved by the Institutional Review Board of the National Institute on Drug Abuse. All participants also received cognitive behavioral therapy. The study took place in Baltimore, MD. Eligible participants were 13–17 years of age, smoked at least ten cigarettes per day (CPD) for the last 6 months, and had a Fagerström Test for Nicotine Dependence (FTND, Heatherton et al., 1991) score of 5 or greater out of 10. Exclusion criteria included pregnancy, recent use of nicotine replacement therapy, untreated acute psychiatric disorder (including drug or alcohol dependence), and lack of parental permission. All participants selected for this study received a medical examination and were in good physical health. Adolescents were accompanied by their parent or guardian on the first visit and written consent for parents and assent for adolescents were obtained.

The sample consisted of 66 adolescent smokers with a mean age of 15.1 years. Girls made up 63.6% of the sample population and European Americans accounted for 66.7% of the sample. The FTND score indicated a high level of dependence, corroborated by the high number of cigarettes consumed per day. Sample demographics and smoking behaviors are provided in Table 1.

2.2. Procedures

Prior to study enrollment, information provided by participants included: demographic data, smoking trajectory, self-reported CPD, and FTND scores. Participants' height and weight were recorded at enrollment. Blood and saliva samples were taken at their first visit to determine cotinine concentrations. An experienced phlebotomist collected blood samples from the antecubital vein to a 7 mL vacutainer tube with lithium heparinate. All samples were iced immediately and centrifuged

Table 1
Sample demographics and smoking behaviors of adolescent smokers ($n=66$)

Baseline measure	Sample mean±SD
Age	15.155±1.3
Gender (% female)	63.60%
Ethnicity (% Euro American)	66.70%
Cigarettes per day	18.3±8.5
FTND Score	7.1±1.3
Body Mass Index	25.2±5.0

for 5 min at 3000 rpm. The plasma was then removed and frozen at −20 °C until the time for assay. A study nurse obtained saliva specimens by asking participants to keep a standard cotton wool dental roll in their mouth for 5 min. Participants were instructed to roll it over their tongue. The dental roll was then placed in a sterile 5 mL two-piece separating tube. All samples were centrifuged for 5 min at 3000 rpm. The top section of the separating tube that contained the dental roll was discarded and the bottom part with extract was stored in a freezer at −20 °C until analysis. Measurements of plasma and saliva nicotine, and its metabolites, cotinine and 3-hydroxycotinine, were performed by Labstat Inc. (Kitchner, Ontario, Canada). Nicotine and cotinine were measured according to the high-resolution capillary-column gas chromatography method described in detail by Teeuwen et al. (1988) and Feyerabend and Russell (1990). Plasma and saliva 3-hydroxycotinine were measured using a capillary gas chromatography–mass spectrometry (GC–MS) method described by Jacob et al. (1992).

2.3. Data analysis

Descriptive statistics were calculated using SPSS 13.0. Pearson correlation coefficients were used to determine the relationship between saliva and plasma levels. The ratio of saliva-to-plasma was computed by dividing individual saliva by individual plasma concentrations. Previous research in adult populations has suggested that individual saliva-to-plasma cotinine ratios are modulated by body mass (Jarvis et al., 2003). Therefore, Pearson correlation coefficients were used to determine the relationship between saliva/plasma ratios and BMI. A partial correlation was used to control for the impact of BMI on the relationship of saliva and plasma concentrations. BMI was calculated using the standard formula: weight (lb) / [height (in)]^2 × 703.

A secondary analysis was conducted to examine correlation differences across gender, ethnicity (European American, African American), and age (younger adolescents age 13–15, older adolescents age >15). In order to control for all relevant variables a multiple regression with plasma cotinine entered as the dependent measure and saliva cotinine, gender, age, and ethnicity as well as the interactions of age *X* saliva cotinine, gender *X* saliva cotinine, and ethnicity *X* saliva cotinine entered as predictor variables was performed in order to determine the relationship between saliva cotinine concentrations and plasma cotinine concentrations in each of the sub-groups. Additionally, *t*-tests were conducted to compare the means of saliva-to-plasma ratios between each of the sub-groups.

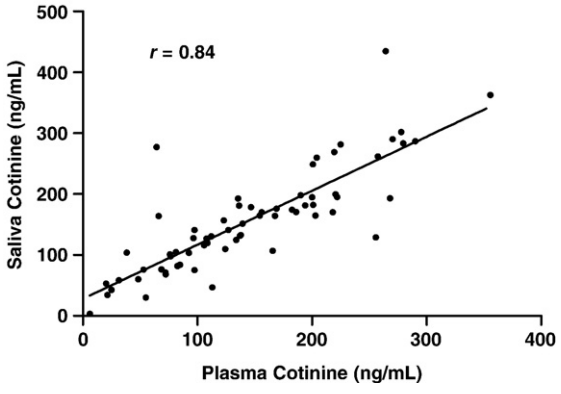

Fig. 1. Scatter plot of saliva and plasma cotinine concentrations for each adolescent smoker (n=66).

Table 2
Mean, median, range and quartile distribution of plasma and saliva cotinine concentrations in adolescent smokers (n=66)

	Plasma (ng/mL)	Saliva (ng/mL)
Median	135.9	146.3
Range	6.2–355.7	3.7–434.8
Quartiles		
25%	80.1	100.4
50%	135.9	146.3
75%	201.5	193.5

3. Results

3.1. Cotinine concentrations

Fig. 1 shows a scatter plot relating saliva and plasma cotinine concentration. The correlation between the two measures was high (r=0.84, p<0.001). Saliva values were on average 11.9% greater than plasma values. The mean ratio of saliva-to-plasma was 1.19. A significant relationship of BMI with the saliva/plasma ratio was found (r=0.41, p<0.001). However, the relationship between saliva and plasma concentration did not significantly change after controlling for BMI in a partial correlation (r=0.846). Mean, median, range and quartile distribution of saliva and plasma cotinine concentrations are displayed in Table 2.

3.2. Cotinine concentration across gender, ethnicity, and age

Table 3 provides mean saliva and plasma cotinine concentration and Pearson correlations by gender, ethnicity, and age. Results of the multiple regression revealed that the predictor variables accounted for a sizable proportion of the variance of plasma cotinine R^2=0.76, F (7, 62)=24.93, p<0.001 (See

Table 3
Means and standard deviations for saliva and plasma concentrations by gender, ethnicity, and age in adolescent smokers (n=66)

	n	Mean age	Saliva (ng/mL)	Plasma (ng/mL)	Pearson's r	S/P ratio
Gender						
Boys	24	15.33	166.55±74.82	152.65±84.11	0.727	1.27
Girls	42	15.02	149.95±89.41	139.31±76.91	0.899	1.15
Ethnicity						
Euro. American	44	15.14	151.86±76.85	143.79±75.30	0.903	1.13
Af. American	19	15.32	170.48±102.92	148.65±92.73	0.737	1.36
Age						
13–15	38	14.21	143.05±85.09	126.97±70.88	0.744	1.26
>15	28	16.39	173.55±81.14	167.50±85.11	0.944	1.10

Table 4
Predictors of plasma cotinine concentrations in adolescent smokers ($n=66$)

Variable	*B*	SE *B*	*β*
Saliva cotinine	0.64	0.13	0.68*
Gender	−13.92	26.81	−0.08
Age	−49.19	24.33	−0.31***
Ethnicity	37.04	25.11	0.21
Age *X* saliva cotinine	0.40	0.13	0.51**
Gender *X* saliva cotinine	0.12	0.15	0.15
Ethnicity *X* saliva cotinine	−0.27	0.14	−0.32

$R^2=0.76$, * $p<0.001$, ** $p<0.01$, *** $p<0.05$.

Table 4 for unstandardized regression coefficient (*B*), standard error, and standardized regression coefficients (*β*) for all predictor variables). Of the control variables only saliva cotinine and age were significant predictors of plasma cotinine ($t=4.77$, $p<0.001$ and $t=-2.02$, $p<0.05$, respectively). The interaction between age and saliva cotinine was significant ($t=3.03$, $p<0.01$) suggesting that older adolescents' saliva cotinine concentrations better predicted plasma cotinine concentrations than that of younger adolescents. The interaction between ethnicity and saliva cotinine approached significance ($t=-1.93$, $p=0.058$) in the direction such that being European American appeared to be a better predictor of plasma cotinine values. The interaction between gender and saliva cotinine did not reach significance. To examine if these results were confounded by cigarettes smoked per a day (CPD), CPD was entered into the model. The results of the interactions did not change. Only the control variable age was affected ($t=1.18$, $p=0.24$). No differences in saliva-to-plasma ratios were observed across sub-groups.

4. Discussion

This study sought to determine the degree of association between plasma and saliva cotinine concentrations in a sample of adolescent smokers. Our findings suggest that in highly-dependent adolescents, cotinine concentrations in saliva are comparable to concentrations found in plasma. This result is consistent with previous research in adult populations (Curvall et al., 1990; Jarvis et al., 2003; Rose et al., 1993). Additionally, the S/P ratio found among adolescent smokers in this study (1.19) is very comparable to that found among adult smokers (1.25) (Jarvis et al., 2003). Furthermore, our findings suggest that there is some variability in degree of correlation between saliva and plasma cotinine concentrations across age and ethnicity. Adolescents older than 15 years exhibited a closer relationship between saliva and plasma cotinine values when compared to younger adolescents. Age-related differences found here support previous findings by Jarvis et al., 2003 in which saliva-to-plasma relationships were lower in individuals under age 50 compared to their older counterparts. Our findings appear to extend these results to adolescents, showing that this relationship continues to weaken as age decreases. Additionally, the saliva-to-plasma correlation among European Americans was marginally greater than that among African Americans. The differences may be attributed to variations in smoking behavior ethnicities. Overall, these findings suggest that there may be higher variability between saliva and plasma cotinine among various sub-groups. Future research should clarify the underlying biological mechanisms of this result as well as how varying smoking behaviors might alter this relationship.

In our adolescent participants, the correlation between plasma and saliva cotinine concentrations was slightly lower than that typically found in adults. One possible explanation for this difference is that salivary glands are continuing to develop through adolescence, with the highest salivary flow rates occurring during this time and young adulthood (Soderling et al, 1993; Yeh et al., 1998). Variations across individuals in the timing of development might lead to a greater range of flow rates in adolescents than in adults. Fenoll-Palomares et al. (2004) found a significant positive correlation between salivary flow rate and pH values. Cotinine is a basic compound whose concentration in saliva is pH dependent (Cone, 1993). This relationship has not yet been assessed in the context of developmental differences in salivary flow rate, but when flow rate is increased experimentally via stimulation, cotinine concentrations decrease by 6% to 26%, presumably due to the accompanying increase in pH (Schneider et al., 1997). Variability in salivary flow rate may be greater in a given sample of adolescents than in a given sample of adults; this variability could explain the difference in our correlation between plasma and salivary levels of cotinine. We did not assess salivary pH and are thus unable to correct for it.

Despite the general association between saliva and plasma cotinine concentration, several limitations must be acknowledged when interpreting these findings. Our sample consisted of heavily dependent smokers which resulted in higher saliva and plasma concentrations than what would have been seen with a wider range of smokers. Also, in our collection of saliva specimens, cotton swabs were used as a collection device rather than a saliva stimulation device. Stimulating saliva samples might help control the salivary flow rate. Also given the recruitment and retention challenges with adolescents, some sub-group sample sizes were small, therefore our results should be replicated with larger sample sizes that include sufficient samples of the sub-groups of interest.

To our knowledge this is the first study to examine the relationship of saliva-to-plasma cotinine concentrations in an adolescent sample. Based on our findings saliva cotinine is generally reflective of plasma cotinine concentration despite some degree of individual variation, we conclude that saliva assay is a low risk option for measuring tobacco use and exposure in adolescents. By avoiding the invasive procedure of venipuncture, researchers may be able to reduce both practical and ethical barriers associated with youths. Future research should consider samples with a broader range of smoke intake and exposure in order to further examine the comparability of saliva and plasma cotinine concentrations across age and ethnicity among adolescents.

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