Validation of Self-Reported Smoking Status Using Saliva Cotinine: A Rapid Semiquantitative Dipstick Method

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Abstract

Purpose: This study evaluated the performance characteristics of a novel rapid method for verifying smoking status in individuals by measurement of cotinine, the primary metabolite of nicotine, in saliva samples using an immunochromatographic strip in a “dipstick” format compared with liquid chromatography/mass spectrometry (LC/MS).

Materials and Methods: A prospective comparison was made of smoking status as determined by measurement of cotinine in urine by LC/MS (the gold standard) and in saliva using a semiquantitative dipstick assay that uses cotinine-specific monoclonal antibodies attached to gold particles and a series of avidity traps to measure cotinine levels (saliva NicAlert®). One hundred seventy-two individuals from a family practice/general medical setting agreed to participate after informed consent and institutional review board approval. Saliva NicAlert® tests were done by untrained operators who followed written directions.

Results: Comparison of smoking status as determined by urine cotinine measurement by LC/MS (50 ng/mL cutoff) with the saliva strip test results, averaged over the two operators, indicated that the saliva test strip results had a sensitivity of 99% and a specificity of 96%. Saliva NicAlert® also identified four smokers who reported being nonsmokers but were confirmed to be smokers by LC/MS.

Conclusions: The saliva NicAlert® assay seems to be a valid, highly sensitive, and specific method for validating self-reported smoking status and may have clinical applications in selected medical settings. (Cancer Epidemiol Biomarkers Prev 2007;16(9):1858–62)

Introduction

The use of tobacco products in general and cigarette smoking in particular is a major public health problem and is the leading preventable cause of disease in our society today. Tobacco use has been identified as a risk factor for cardiovascular diseases, lung and other cancers, chronic respiratory diseases, stroke, and complications of pregnancy (1-4). Annually, an estimated 438,000 people in the United States (5) and 4.9 million people worldwide (6) die prematurely as a result of tobacco use. Another 50,000 die each year as a result of exposure to second hand smoke [environmental tobacco smoke (ETS)]. Despite the public awareness of the health risks tobacco use poses, ~21.6% (45.4 million) adults in the United States currently smoke cigarettes (7). The annual health care costs attributable to cigarette smoking in the United States have been estimated at $75.5 billion and the attributed annual productivity losses at $92 billion (5).

Reducing tobacco use and dependence has been identified as a key strategy in reducing the significant long-term health effects and associated economic costs of tobacco use. Clinical practice guidelines recommend the consistent identification and documentation of tobacco users in the health care setting as the first step in clinical interventions to counsel and treat tobacco users (8-11), and it has been recommended that determining smoking status should be a vital sign (8, 12-14). Self-reported smoking status helps to identify a significant percentage of smokers but does not identify all smokers. Data suggests that a percentage of patients ranging from 1.4% in broadly based epidemiologic studies to as high as 35% in populations where smoking is a known risk factor, such as patients with respiratory disease (18%), cancer patients (20%), and pregnant women (35%) will self-report inaccurately due to a variety of factors (such as misunderstanding, intentional deception, embarrassment, denial, shame, etc.; refs. 15-20).

Measurement of cotinine, a primary metabolite of nicotine that has a half-life of 16 to 18 h and that can be detected in urine, saliva, or serum, provides a reliable means of determining smoking status and other tobacco product use or exposure over a period of 2 to 3 days (20-22). For smokers, another method of determining tobacco use is expired carbon monoxide. A relatively short half-life of 4 h limits the reliability and accuracy of detecting smokers using expired carbon monoxide testing, and carbon monoxide testing is unable to detect the use of smokeless tobacco (chew, dip, or snuff products). At this time, cotinine measurement has not been routinely used in clinical settings mainly due to the time involved, the methods required to collect the sample, and the cost...
and inconvenience of sending samples to laboratories. However, the validation of reported tobacco use status may have significant clinical importance in cessation clinics, research settings, pulmonary and pediatric clinics, or transplant candidate selection and may have an effect on the determination of insurance premiums.

This report evaluates a new rapid method for measuring cotinine levels using saliva samples to verify smoking status in individuals in a clinical setting, such as a family practice or other general medicine setting. An immunochromatographic test strip in “dipstick” format (saliva NicAlert®) was used to measure cotinine levels in saliva samples. The sensitivity and specificity of the urine dipstick NicAlert® method was previously independently validated by several groups with urine NicAlert® results being found to compare favorably to the ELISA laboratory method and to the mass spectrometry method (23-25). The ease and convenience of providing a saliva sample may make a saliva-based test more preferable in a clinical setting than a urine-based test.

In this study, a prospective comparison was made between self-reports of smoking status and a semi-quantitative, enzyme-linked, immunosorbent assay-based method (saliva NicAlert®) testing for the presence of cotinine. Results were validated by urine liquid chromatography/mass spectrometry (LC/MS) by an independent reference laboratory.

Materials and Methods

Subjects. One hundred seventy-two individuals participated from a family practice/general medical setting after informed consent and institutional review board approval. Subjects were primary care outpatients at two different sites. Five patients (2.9%) were excluded from the final analysis: in three (of 172) cases, there was an inadequate amount of saliva provided by the subject, and in two (of 172) cases the operator was unable to report a reading from the strip (presumed to be due to technique or mishandling). Of the analyzed group (n = 167), 36.5% (n = 61) were men and 63.5% (n = 104) were women; for two subjects, sex was not recorded. The mean age of subjects was 42.1 years with the youngest subject being 6 years old and the oldest was 80 years old. The overall prevalence of current self-reported smoking in the group was 46.1% (n = 77). The characteristics of the study population are summarized in Table 1.

Table 1. Characteristics of study population

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (y)</td>
<td>N = 167*</td>
<td>n = 61</td>
<td>n = 104</td>
</tr>
<tr>
<td></td>
<td>42.1</td>
<td>41.6</td>
<td>42.3</td>
</tr>
<tr>
<td>Range of ages (y)</td>
<td>6-80</td>
<td>6-68</td>
<td>12-80</td>
</tr>
<tr>
<td>Self-reported smoking status</td>
<td>Smoker</td>
<td>77 (46.1%)</td>
<td>25 (41.0%)</td>
</tr>
<tr>
<td></td>
<td>Nonsmoker</td>
<td>90 (53.9%)</td>
<td>36 (59.0%)</td>
</tr>
</tbody>
</table>

*Sex of two subjects was not noted.

Results

The saliva NicAlert® cutoff for smoker versus nonsmoker is 1. Results determined by NicAlert® (≥1) indicates that the subject is a smoker or has significant exposure to ETS. Based on a comparison between the reported results for the two untrained operators at each site, the overall agreement in determining smoking status was 96.3% at site 1 (κ = 0.92, 95% confidence interval 0.71-1.00, P < 0.0001) and 98.7% at site 2 (κ = 0.97, 95% confidence interval 0.75-1.00, P < 0.0001). The data in Table 2 display the sensitivity of saliva NicAlert® to detect smokers as determined by LC/MS based on a 50 ng/mL cutoff was 100% for three of the four operators and 96.7% for the fourth operator. The specificity of saliva NicAlert® to detect nonsmokers as determined by LC/MS ranged from 100% for one operator (no false positives) to 91.8%.
chewing tobacco and smoking cigarettes. Of the reported smokers, 3 reported light use (half pack a day or pipes and cigars), 19 reported smoking half pack of cigarettes a day, 36 reported one pack, 8 reported one and a half packs, and 3 reported two packs. Three reported nonsmokers had LC/MS readings of >200 ng/mL, indicating likely deception. One reported nonsmoker reporting ETS exposure of 5 to 8 h at work and 9 to 12 h at home had a urine LC/MS reading of 86.1 ng/mL. A second reported nonsmoker with reported ETS exposure of 9 to 12 h at home had a urine LC/MS reading of 30.6 ng/mL; all other reported nonsmokers (79 of 84 or 94%) had urine LC/MS values of <20 ng/mL, with 74 of 84 or 88% having urine LC/MS values of <10 ng/mL.

Reference laboratory testing of urine cotinine by LC/MS, using a cutoff of 50 ng/mL, identified 98.7% (76 of 77) of the self-reported smokers. The one (of 77) false negative was an individual who self-reported smoking one to two pipes/cigars a day. The saliva NicAlert results also reported this individual as a nonsmoker. LC/MS identified four reported nonsmokers as smokers based on the urine cotinine levels determined by LC/MS. Saliva NicAlert also was able to identify the same four self-reported nonsmokers as smokers based on their NicAlert readings. Three of these individuals identified were clearly “deceivers,” with cotinine values ranging from 202 to 1051 ng/mL; the fourth was a likely deceiver with a cotinine value of 86 ng/mL but also reported significant ETS exposure at work, home, and socially. No LC/MS results were reported for six subjects, and the individual operators were not able to provide results for seven strips (7 of 322 or 2.1%).

Discussion

The use of an easy to obtain, reliable, highly sensitive, and specific assay for tobacco use status that can be done by untrained personnel at the point of care should have a

Table 2. LC/MS determination of smoking status (50 ng/mL cutoff) versus urine NicAlert determination for the two operators at each site

<table>
<thead>
<tr>
<th>LC-MS Site 1</th>
<th>Operator 1</th>
<th>Operator 2</th>
<th>LC-MS Site 2</th>
<th>Operator 1</th>
<th>Operator 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-smoker (NicAlert = 0)</td>
<td>47</td>
<td>2</td>
<td>45</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Smoker (NicAlert ≥ 1)</td>
<td>0</td>
<td>32</td>
<td>1</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>95.9%</td>
<td>96.7%</td>
<td>91.8%</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: No LC/MS results were reported for six subjects, and the individual operators were not able to provide results for seven (of 322) strips (2.1%).

Figure 1. The NicAlert strip.
Table 3. Summary of LC/MS values in reported smokers versus reported nonsmokers

<table>
<thead>
<tr>
<th>Status</th>
<th>LC/MS, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-reported smoking status</td>
<td>Mean* 29.3</td>
</tr>
<tr>
<td>Reported nonsmokers (n = 84)</td>
<td>SD* 149.6</td>
</tr>
<tr>
<td>Minimum Reading</td>
<td>BLQ (&lt;1)</td>
</tr>
<tr>
<td>Maximum Reading</td>
<td>1051.1</td>
</tr>
<tr>
<td>Reported smokers/ users (n = 77)</td>
<td>Mean* 1851.9</td>
</tr>
<tr>
<td>Minimum Reading</td>
<td>8.8</td>
</tr>
<tr>
<td>Maximum Reading</td>
<td>ULOQ (&gt;8000)</td>
</tr>
<tr>
<td>Total subjects (N = 161)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ULOQ, upper limit of quantitation; BLQ, below limit of quantitation.

*Reported below limit of quantitations treated as a reading of 1 ng/mL.

The saliva dipstick methodology has potential application in a clinical setting to objectively validate the assessment of smoking status or exposure to smokeless tobacco use and to help guide interventions, such as counseling for cessation, detection of relapse, or the need to reduce environmental tobacco exposure. A semiquantitative saliva assay has the advantage in the clinic of being able to provide immediate feedback without the need for interruption or leaving the room and returning later (e.g., urine assay) or mailing to a reference laboratory (e.g., blood or urine assays). The more immediate test result could improve the value from significant role in many clinical and research settings when the accurate assessment of tobacco use or exposure is required. The present study has the limitations that it was undertaken in a family medicine/general practice population and did not include significant numbers from obstetric, infant, or geriatric populations. However, the NicAlert® assay would be expected to perform as reliably with any of these groups. Further research to validate this theory may be required. The percentage of reported smokers in the study (46%) was greater than that reported for the general population in the two states where the study was conducted (25.7%, Tennessee 2005; 27.4%, West Virginia 2005 (27)).

The urine-based strip is more practical for the detection of ETS exposure because of the higher levels of cotinine in urine compared with saliva in the same individual. From a research perspective, it is widely recognized that studies evaluating disease outcomes related to smoking and cessation rates should incorporate the most sensitive and specific objective measurement for confirming self-report. Misclassification may affect efficacy data in clinical trials. Calculations of smoking-related risk in both smokers and nonsmokers may be affected, depending on study size. The use of the dipstick saliva device simplifies the measurement procedure and may be helpful in research studies where smoking status or ETS exposure is an inclusion or exclusion criterion or a variable to be controlled for in the research analysis. Furthermore, unlike a urine test, a saliva sample can easily be witnessed.

Stopping teenagers from initiating or continuing to smoke remains a high public health priority. The validation of tobacco use status using easily obtainable saliva samples may also be appropriate for pediatric or adolescent patients where early detection of tobacco use may result in early intervention, improved health, and a reduction in acute or chronic illness, such as asthma. Although the majority of chronic diseases that result from the effects of tobacco use occur later in life, most current smokers start smoking in their teens, and tobacco is associated with other high risk behavior, including illegal drug use (28).

Although it is feasible that this saliva method could be used to identify minors exposed to ETS, it would mainly be applicable to individuals with heavy exposure to ETS. The urine-based strip is more practical for the detection of ETS exposure because of the higher levels of cotinine in urine compared with saliva in the same individual. In summary, there is a need in some clinical and research settings for a simple, on-site test to verify tobacco use status or exposure to tobacco smoke. The saliva NicAlert® test was able to distinguish tobacco users from nonusers in a clinical setting with high degree of accuracy using LC/MS as the reference standard. The
saliva dipstick has many potential applications in clinical settings as part of the routine assessment of smoking status as a vital sign and to help guide clinical interventions and risk management. In research settings, this methodology for tobacco use or exposure could improve patient selection, data collection, and analysis. For insurance companies, tobacco use may influence premium rates. However, the attraction of this and other cotinine detection or smoking status tests will depend on test costs, methods and amounts of reimbursement for performing the tests, and clinicians' experience of the utility and value of such testing in a clinical setting.

Acknowledgments
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References
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