Urine Cotinine as an Index of Smoking Status in Abstinent Smokers: Comparison of GC/MS with Immunoassay Test Strips

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Biomarkers such as carbon monoxide (CO) and cotinine (nicotine metabolite) are used in cessation studies to assess smoking status. CO is easy to assess, inexpensive, and provides immediate results. However, CO's short half-life may limit its ability to identify smokers who have abstained for several hours. Quantitative methods (e.g., GC/MS) for measuring urine cotinine, which has a longer half-life, are valid and reliable, though costly and time-consuming. Recently developed semi-quantitative urine cotinine measurement techniques (i.e., urine immunoassay test strips or ITS) address these disadvantages, though the value of ITS as a means of identifying abstaining smokers has not been evaluated. The purpose of the present study was to examine ITS as a measure of smoking status in abstaining smokers. A total of 236 breath and urine samples were collected from smokers who participated in two separate studies involving three independent, 96-hour (i.e., Monday-Friday), Latin-square ordered, abstinence or smoking conditions; a minimum 72-hr washout separated each condition. Each urine sample was analyzed with GC/MS and ITS. There was a moderate and significant linear relationship between CO and GC/MS ($r^2 = 0.37$), and a strong and highly significant exponential relationship between ITS and GC/MS ($r^2 = 0.71$). Under these study conditions, ITS assessment showed strong sensitivity (98.5%) and weak specificity (58.5%). ITS may be most valuable for identifying current smokers. Validation of ITS using GC/MS results from smokers undergoing >96-hour abstinence may be valuable, especially if ITS is used for verification of smoking status in cessation trials/programs.

Introduction

Biomarkers such as carbon monoxide (CO) and cotinine (nicotine metabolite) are used in cessation studies to assess smoking status. These biomarkers are valuable because they are more accurate then self-report, especially in circumstances where smokers perceive pressure to achieve abstinence (Gilbert, 1993; Jarvis, Thunstall-Pedoe, Feyerabend, Vesey, & Saloojee, 1987; Wagenknecht, Burke, Perkins, Haley, & Friedman, 1992). CO is frequently used because it easy to assess, inexpensive, and provides immediate results. It also has shown acceptable sensitivity and specificity (about 90%) when discriminating smokers from non-smokers (Benowitz, 1983; Jarvis et al., 1987). However, a metabolic half-life of 4 hours may limit the usefulness of CO for detecting smoking status in smokers who report abstinence, as they may change their smoking behavior to appear abstinent (Gariti, Alterman, Ehrman, Mulvaney, & O'Brien, 2002).

Cotinine, with a half-life in urine of 20 hours (Jarvis et al., 1987), may be a better measure of smoking status, given its superior sensitivity and specificity relative to CO (Gariti et al., 2002; Murray, Connet, Istvan, Nides, & Rempel-Rossun, 1993). Quantitative methods for measuring cotinine (e.g., GC/MS) are valid and reliable, though they are also costly, time-consuming, and require special equipment and personnel (Jarvis et al., 1987). Recently developed semi-quantitative urine cotinine measurement techniques (i.e., urine immunoassay test strips or ITS) address these disadvantages: they are less expensive, immediate, and require no special equipment/personnel. In studies examining smokers and non-smokers, ITS sensitivity (i.e., ability to identify smokers correctly) and specificity (ability to identify non-smokers correctly) has been validated with other biomarkers, with mixed results. For example, compared to more quantitative measures (i.e., GC/MS), some studies have reported that ITS has moderate sensitivity (i.e., 72.3%) and specificity (i.e., 70.4%; Karnes, et al., 2001), while others have been

more positive for sensitivity (97.3%, specificity = 74.5%; Parker et al., 2002), or sensitivity (90.5%) and specificity (90.6%; Gariti et al., 2002). However, no research has validated the use of ITS for detecting smoking status in recent or former smokers.

In some areas of nicotine and tobacco research (e.g., cessation studies), identifying smokers who actually stop smoking during a period of attempted abstinence is critical. This clinically relevant task can be more difficult than identifying individuals as smokers or non-smokers, because, in abstaining smokers, CO and cotinine decrease over time. Thus, any method of identifying abstinent smokers must be sensitive to time-dependent changes in biomarker levels. The purpose of the present study was to examine ITS as a measure of smoking status in smokers during a 96-hour period of attempted abstinence. We assessed the relationship of ITS to quantitative measures of urine cotinine level (i.e., GC/MS), and calculated the specificity and sensitivity of ITS (using GC/MS as a reference criterion) as a means of identifying abstinent and non-abstinent smokers. In addition to examining ITS, we also compared ITS to CO, to examine whether or not this new technology can improve upon an already existing inexpensive, easy-to-use biomarker of smoking status. Thus, this study is the first to compare CO and ITS with GC/MS in smokers during a period of attempted abstinence.

Method

Setting and participants

A total of 44 men and women participated in two separate IRB approved studies (Buchhalter et al., 2002; Breland et al., 2003). Participants were included if they were 18-50 years old (mean = 24.5, SD = 8.0), provided a breath sample $\geq$15 parts/million CO at screening (mean = 25.9, SD = 11.7), and smoked $\geq$15 king-sized cigarettes/day (mean = 19.8, SD = 2.7). They were moderately nicotine dependent, as indicated by the Fagerstrom (1978) nicotine

tolerance questionnaire (Mean = 4.9, SD = 0.9). Exclusion criteria included past or current cardiovascular disorders, current pregnancy, breastfeeding, or smoking cessation or reduction efforts. As described below, the present analyses involve 236 urine samples and expired-air CO measurements across these two studies.

Urine sample and CO measurement procedure

Participants in each study completed three, Latin-square ordered, 5-day conditions (Mon-Fri). In the first study (Buchhalter et al., 2002) data from two conditions are included here: one in which participants smoked cigarettes containing nicotine (see Pickworth et al., 1999) and one in which they were instructed to abstain from smoking (the third condition involved smoking denicotinized cigarettes). In the second study (Breland et al., in press), data from all three conditions are included here: two in which participants smoked cigarettes containing nicotine (own brand or Advance™, see Breland et al., 2002; in press) and one in which they were instructed to abstain from smoking. For both studies, participants smoked their own brand of cigarettes between conditions (minimum of 48 hours). Breath samples were tested for CO level on days 1-5 (BreathCO, Viatalograph Inc., Lenaxa, KS). Urine samples were obtained on days 1, 3, and 5. Semi-quantitative urine cotinine was assessed immediately (using Nicalert® test strips; Nymox Corp., Maywood, NJ), and 3-ml aliquots were stored at -70^{o}C for later quantitative cotinine analysis. On days 3 and 5, CO and semi-quantitative urine cotinine data were used to assess compliance with condition smoking restrictions. For example, when participants were instructed not to smoke, compliance was verified with decreases in CO and semi-quantitative urine cotinine, relative to day 1. CO was measured in parts per million (ppm) while ITS values of 0-6 were obtained by a trained experimenter reading the ITS strip. Compliance was reinforced monetarily in each study (i.e., $30 on day 3 and $70 on day 5).

Participants who failed to comply with condition restrictions once were offered another chance to complete the condition. Participants who failed to comply with condition restrictions more than once were withdrawn from the studies. After each study was completed, urine samples were analyzed for cotinine level (GC/MS; LOQ = 5 ng/ml; modified from Jacob et al., 1991). Due to financial constraints, samples from day 1 and 5 were analyzed from study 1 (i.e., a total of 128 samples). All 108 samples were analyzed from study 2.

Data Analysis

All CO and ITS data were submitted to a regression analysis as predictor variables, with GC/MS cotinine level as the criterion variable. In addition, sensitivity and specificity of CO and ITS were obtained, using GC/MS urine cotinine as the reference criterion (<100 ng/ml defined an abstinent smoker; Gariti et al., 2002; Parker et al., 2002).

Results

Figure 1 presents the results of the regression analyses for CO and ITS, using GC/MS cotinine level as the criterion variable. Both predictors yielded positive statistically significant relationships, though the relationship between ITS and GC/MS was much stronger. CO demonstrated a linear relationship with GC/MS ($r^2 = 0.37$; $p < .01$), while ITS demonstrated an exponential relationship with GC/MS ($r^2 = 0.71$; $p < .01$). For both biomarker predictors, as levels of CO or ITS increased, so did GC/MS cotinine level.

< Insert Figure 1 about here>

<Insert Table 1 about here>

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Tables 1 and 2 show the number of samples identified by GC/MS as indicating abstinence (< 100 ng/ml) or non-abstinence that were also identified by CO (Table 1) or ITS

(Table 2) as coming from abstinent or non-abstinent smokers. For CO, we used < 8 ppm as an indicator of abstinence (e.g., Heil, Tidey, Holmes, Badger, & Higgins, 2003) while for ITS we used < 3 as an indicator of abstinence (as described in the package insert). CO was perfect in correctly identifying samples collected during GC/MS verified abstinence (specificity = 100%), though this measure sometimes misidentified samples collected during GC/MS verified non-abstinence as having been collected during abstinence (sensitivity = 83.1%). In contrast, ITS often misidentified samples collected during GC/MS verified abstinence as having been collected during non-abstinence (specificity = 58.5%). However, ITS was near-perfect in correctly identifying GC/MS verified non abstinence (sensitivity = 98.5%). For ITS, the cause for the poor specificity was a high level of false positives (41.5%), indicating that ITS results were greater than 3 when, according to GC/MS, they should have been lower.

Discussion

The current study was the first to compare two quick and cost-effective biomarkers (CO and ITS) with GC/MS as a means for assessing smoking status in smokers undergoing a 96-hour period of attempted abstinence. Results indicate significant relationships between both biomarkers and quantitative cotinine, with a stronger relationship observed between ITS and GC/MS. Limitations were observed for both biomarkers in this study. For example, while CO was very effective for identifying samples collected from smokers who were actually abstaining, it was less successful at identifying non abstinence. CO classified 17% of samples as having come from participants who were abstaining when, according to GC/MS, these samples came from smokers who had continued to smoke. ITS demonstrated an excellent ability to identify samples that came from smokers who were currently smoking, but a poor ability to identify correctly samples that came from smokers who were currently abstaining. In fact, in spite of its

strong relationship to quanitative cotinine, ITS classified as non-abstinent nearly half of the samples collected from smokers who were abstinent, according to GC/MS.

The current results are consistent with previous research addressing the relationship between CO and GC/MS verified smoking (Niebala et al., 2002; Gariti et al., 2002). One challenge in relying solely on CO to verify self-reported smoking status is that, similar to the current findings, CO is often unable to identify smokers who abstain for several hours before providing a breath sample. For these smokers, CO may be an insufficient verification tool when abstinence is required and/or rewarded.

Given the challenge CO presents in verifying abstinence, and the fact that cotinine is a superior measure (Jarvis et al., 1987; Murray et al., 1993; Gariti et al., 2002), cotinine assessment that is easy, cost-effective, and immediate may be an important verification tool. ITS is easy and immediate, and relative to GC/MS, can be cost-effective. Data from the current study suggest that ITS may be an excellent tool for identifying smokers who abstain for several hours before providing a urine sample. Unfortunately, the current study also suggests that ITS may have limited utility for verifying short-term periods of smoking abstinence (i.e., 96 hours). One potential explanation for this limitation is that these ITS strips measure cotinine and trans-3'hydroxycotinine (Dr. M. Munzar, Nymox Corp., personal communication), a cotinine metabolite (Benowitz & Jacob, 2001). Thus, when cotinine levels are low after a four day period (Jarvis et al., 1988), trans-3'hydroxycotinine levels can remain high, and may then yield a value of 3 or greater on the ITS strip (i.e., indicating non-abstinence). Similar studies with a longer abstinince period (i.e., greater than 96 hours) and/or concurrent GC/MS measurement of trans-3'hydroxycotinine may provide a more complete determination of ITS utility as an index of smoking status.

In summary, this study was the first to examine quick and cost-effective biomarkers (CO and ITS) used to assess smoking status in abstinent and non-abstinent smokers. Results suggest that there are limitations associated with ITS, in its current form, when examining short-term abstinence. These strips should be evaluated in smokers undergoing longer-term abstinence to determine their clinical usefulness. ITS strips that measure cotinine but not trans-3'hydroxycotinine may be particularly valuable for verification of short-term abstinence.

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Figure Caption

Figure 1. The relationship between expired-air CO and GC/MS urine cotinine (left panel) and ITS and GC/MS urine cotinine (right panel) for 236 breath and urine samples collected from 40 smokers during periods of smoking and attempted abstinence. Trend lines were fitted with least squares regression model. The trend line for CO and GC/MS is linear while the trend line for ITS and GC/MS is exponential.

Table 1. Comparison of expired-air CO to GC/MS

	GC/MS Cotinine	
Expired-air CO	101+	0-100
8+	162	0
0-7	33	41

Specificity = 100.00%
Sensitivity = 83.08%

Table 2. Comparison of ITS to GC/MS

	GC/MS Cotinine	
ITS	101+	0-100
3+	192	17
0-2	3	24

Specificity = 58.54%
Sensitivity = 98.46%