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Using NicAlert Strips to Validate Smoking Status Among Pregnant Cigarette Smokers

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Abstract

Background—Decreasing smoking during pregnancy is an important public health priority. An important step towards decreasing smoking during pregnancy is wider dissemination of evidence-based smoking cessation interventions. One such intervention is contingency management wherein mothers earn vouchers exchangeable for retail items contingent on biochemically-verified smoking abstinence. Wider dissemination may be possible by using smoking verification methods that require minimal training and equipment. One possibility is to use a cotinine-sensitive dipstick (NicAlert) rather than a bench-top cotinine analyzer, which is expensive and requires relatively extensive technician expertise, or breath carbon monoxide analysis, which is relatively nonspecific. The present study was conducted to begin examining the utility of cotinine-sensitive dipsticks for this purpose.

Methods—Fifty urine samples from pregnant women enrolled in a smoking cessation program were analyzed to compare three different methods for verifying smoking status: NicAlert strips, a bench-top enzyme multiplied immunoassay technique (EMIT) analyzer, and gas chromatography (GC), the current gold standard for determining cotinine levels in urine.

Results—Agreement between GC and NicAlert results were high (96%) and comparable to agreement between GC and EMIT results (94%). Semi-quantitative measurements using NicAlert were low with only 30% of samples in agreement between GC and specific ranges given on the strips.

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Conclusions—NicAlert strips appear to be a valid measure of determining smoking status among pregnant smokers although not of absolute cotinine concentration. With minimal training and equipment required, NicAlert strips provide a potentially practical method for using urine cotinine to verify smoking status in community treatment settings.

Keywords

NicAlert; urine cotinine; pregnant smokers; gas chromatography; smoking status; biochemical verification; contingency management

1. Introduction

Smoking is the leading preventable cause of poor pregnancy outcomes in the U.S. (Bonnie et al., 2007) and development and dissemination of more effective smoking-cessation interventions for pregnant women is an important public health priority (U. S. Centers for Disease Control, 2010). One such treatment approach with demonstrated efficacy is contingency management (CM) wherein women earn vouchers exchangeable for retail items or other financial incentives contingent on biochemically-verified smoking abstinence (Donatelle et al., 2000; Heil et al., 2008; Higgins et al., 2004; Higgins et al., 2010). In randomized clinical trials demonstrating the efficacy of this approach, smoking status was objectively verified at each clinic visit by measuring urine cotinine levels with an onsite bench-top analyzer that used a semi-quantitative enzyme multiplied immunoassay technique (EMIT). Compared to the gold-standard analysis method, gas chromatography (GC), EMIT tests provide nearly identical results (≥98% agreement) in terms of smoking status classification (Higgins et al., 2007).

Unfortunately, while less expensive than GC, EMIT testing is more expensive, and requires more technical expertise, than community smoking-cessation clinics who might adopt this intervention would likely be able to support. Indeed, voucher-based CM for smoking during pregnancy has been disseminated to numerous clinics throughout the United Kingdom (Ballard and Radley, 2009), but breath carbon monoxide (CO) testing is being used in place of cotinine testing. While breath CO is effective at objectively discriminating smokers from non-smokers, it is associated with a substantially greater error rate (false negatives) than cotinine testing (Higgins et al., 2007). This greater error rate can be attributed to the relatively short half-life of CO, (~2–8 hours; Benowitz et al. 2002) which allows infrequent or low levels of smoking to go undetected, and the possible contamination of results via CO sources other than cigarette smoking. Comparatively, cotinine has a longer half-life (~16–20 hours; Benowitz et al. 2002) and is less susceptible to non-smoking influences, making it a more effective biomarker for classifying smoking status. However, the greater costs and need for technical expertise likely precludes using bench-top immunoassay techniques to verify smoking status in community-based smoking-cessation interventions.

One potential alternative is semi-quantitative immunochromatographic assay test strips (e.g., NicAlert, Nymox Pharmaceutical Corporation, Montreal, Quebec) which are commercially available for the measurement of urine cotinine levels. NicAlert test strips are dipsticks that require no special equipment and minimal training. Researchers have already begun to use NicAlert strips in CM interventions. For example, Cavallo et al. (2007) tested a CM intervention with adolescent cigarette smokers where smoking status was confirmed using CO levels and NicAlert strips. End-of-treatment abstinence rates were confirmed by GC testing. Unfortunately, the researchers did not report how the GC results compared to NicAlert strip results. Other researchers have made comparisons between NicAlert and GC results (Acosta et al., 2004). However, to our knowledge, no one has reported how NicAlert would perform with pregnant smokers. Because pregnant women metabolize nicotine more

rapidly than non-pregnant populations (Dempsey et al., 2002) results from previous studies might not generalize to pregnant smokers.

The purpose of the present study was to examine the specificity and sensitivity of NicAlert test strips in classifying the smoking status of pregnant smokers receiving a smoking-cessation intervention, using GC as a reference criterion. In addition, the study presented an opportunity to determine agreement between NicAlert test strips, bench-top EMIT, and GC measures of smoking status in this population.

2. Methods

2.1 Participants

Data were obtained from pregnant smokers enrolled in a university-based outpatient research clinic for smoking cessation. All participants were recruited from obstetric practices in the greater Burlington, Vermont area. Participant characteristics at the intake assessment are outlined in Table 1.

2.2 Procedure

Women completed assessments of smoking status at 28-weeks antepartum and 12- and 24-weeks postpartum including providing a urine sample for measuring urine cotinine levels. Each urine sample was divided into three parts for testing purposes. One third was analyzed immediately using onsite bench-top EMIT. The second third was frozen and shipped at approximately monthly intervals to LabStat International (Kitchener, Ontario, Canada) for quantitative urine cotinine testing using well-established GC methods. The remaining third was frozen and retained for future use, including use in the current study.

2.3 Dipstick training

Testing with NicAlert test strips was performed in our research laboratory. Scorers were initially trained by testing 10 urine samples not included in the present study. Urine samples were brought to room temperature and NicAlert test strips were dipped into a cup containing 2ml of the urine for 20 seconds. They were removed and placed on plastic “testing area” card per the manufacturer’s instructions. After 20 minutes, two independent scorers identified the level on the strip. The test strips have 6 levels (0–6) corresponding to escalating levels of urine cotinine (see Table 2). The result of the test strip is identified by locating the lowest level where a red band appears. Inter-rater reliability for the training samples was 100%; the ranges identified on each sample (0–6) were scored identically by both scorers.

2.4 Dipstick testing

For the present study, 50 urine samples were selected randomly from the 28-week antepartum assessments. Testing of the samples was conducted as described above. Both scorers were blind to the GC and EMIT results of each sample. As recommended by the manufacturer, a NicAlert test strip score of 3 (100–200ng/ml) or higher indicated positive smoking status. For GC results, a 50ng/ml cut-off was used (Society for Research on Nicotine and Tobacco’s Subcommittee on Biochemical Verification, 2002), however using a cut-off of 25ng/ml produced identical results. For EMIT testing, a cutoff of 80 ng/ml was used, as verified for use by previous studies with pregnant smokers (Higgins et al., 2007). Analyses examined both NicAlert strips’ ability to discriminate between smokers and non-smokers, and the accuracy of its semi-quantitative results.

3. Results

3.1 Inter-rater reliability

Inter-rater reliability was high (96%, 48/50) using the test strips. For the two tests where there were disagreements between raters, samples were scored as a 5 by one rater and a 6 by the other. Both scores indicate positive smoking status, and therefore did not affect the rest of the analyses.

3.2 Sensitivity and specificity

The relationship between NicAlert test strips results and GC results were examined to determine the accuracy of the former in identifying positive smoking status (i.e., sensitivity) and negative smoking status (i.e., specificity) (Table 2). Sensitivity (94.12%, 32/33) and specificity (96.97%, 16/17) were high and similar to EMIT results. Agreement between GC and NicAlert was 96% (48/50), nearly identical to the agreement between GC and EMIT, 94% (47/50).

3.3 Semi-quantitative results

NicAlert strips did not perform as well when used semi-quantitatively. Only 30% of the samples were in agreement between GC results and the ranges provided by the NicAlert strips. NicAlert results tend to overestimate cotinine levels as compared to GC results (Table 2). Long periods of abstinence did not improve semi-quantitative results in this population. In the 15 samples where subjects had been abstinent for more than 5 days only one sample had the correct semi-quantitative result as determined by GC.

4. Discussion

Overall, NicAlert strips are quite accurate at discriminating pregnant smokers from abstainers. The strips require minimal training for use and interpretation, no additional equipment, and results are available in 20 minutes. These strips could prove useful for smoking-cessation programs for pregnant smokers where accurate classification of smoking status is a priority, but purchase or upkeep of immunoassay or GC equipment/services are not feasible and/or where obtaining quantitative or semi-quantitative results is not required.

Additionally, NicAlert strips have the potential for diverse clinical utility. Similar products (e.g., NicoMeter cotinine dipsticks) have been used to classify pulmonary patients' smoking status (Gariti et al., 2002), in CM treatments for smoking cessation with adult and adolescent smokers (Schepis et al., 2008) and in testing self-reported smoking status of hospital staff (Parker et al., 2002).

Another version of NicAlert strips which test saliva cotinine could also further proliferate dipstick use. Cooke et al. (2008) measured the accuracy of NicAlert saliva tests in a group of men and non-pregnant women (averaging more than 10 cigarettes per day). Compared to GC, the saliva dipstick had a sensitivity of 93% and a specificity of 95%. These rates are not only comparable to rates obtained testing urine but testing saliva may also be more acceptable in some circumstances. Validation of the effectiveness of the test strips for saliva should be examined with pregnant women prior to use in treatment.

NicAlert results are most accurate when abstinence has been maintained for at least 5 days, which is true for any use of cotinine to verify abstinence due to cotinine's relatively long half-life (Acosta et al, 2004; Schepis et al 2008). However, NicAlert strips may be sensitive to other nicotine byproducts in addition to cotinine. For example, Acosta and colleagues (2004) compared results using NicAlert and GC when testing smokers (averaging 20

cigarettes a day) after 96 hours of abstinence. They found that when NicAlert strips were used during the first 96 hours of abstinence sensitivity was high (98.5%) but specificity was low (58.5%). However, specificity increased the longer abstinence was maintained. Acosta and colleagues (2004) suggest that the NicAlert strips may also be sensitive to trans-3′hydroxycotinine, which may explain the low specificity during the initial days of abstinence. A lack of specificity was not seen in the current data set, perhaps due to the fact that the women in this study had been attempting to abstain for longer than a week at this point.

To overcome issues of initial specificity, researchers can define abstinence during the first week differently than in the following weeks. For example Higgins et al. (2004) and Heil et al. (2008) used once-daily CO monitoring to verify abstinence during the initial quit week among pregnant smokers (averaging 20 cigarettes/day before and 10 cigarettes/day after they found they were pregnant). During the subsequent weeks cotinine levels were used to determine smoking status. Using CO during the first week of the quit attempt allows for clearance of cotinine from smoking prior to the quit date and permits its use as an accurate marker of smoking status in later weeks. Additionally, other researchers have defined abstinence during the first week as decreasing levels of cotinine rather than a strict cut-off point (Cavallo et al., 2007).

Overall, the results from this study are very similar to other studies done using NicAlert in non-pregnant, heavier smoking populations (Cooke et al., 2008). NicAlert performs well when discriminating smokers from nonsmokers and overestimates cotinine concentration in semi-quantitative assessment. However, even in this pregnant (and thus presumably faster cotinine metabolizing) population, who are currently smoking fewer than 10 cigarettes per day on average, cotinine level is still being over-estimated even with long periods of abstinence, as is evidenced by the semi-quantitative results. As such, caution is recommended when interpreting semi-quantitative results when using NicAlert.

Regarding potential limitations, this study consisted of only 50 samples. A larger sample size may have revealed problems not discerned in this modest sample. Another potential limitation for NicAlert strips is that their cost can appear relatively high (Bernert et al., 2005). However, if directly compared, the costs of bench-top EMIT and NicAlert testing is very similar, and NicAlert testing can even be more cost effective if small numbers of samples are being analyzed. In an estimated direct cost comparison with EMIT testing, if purchased in units of a hundred or more, NicAlert strips can cost $10.25 per sample. The per sample cost of analyzing samples using an EMIT machine such as a VIVA E or Microgenics MGC240 is about $7.00– $8.00. This price estimate includes costs of reagents and lab supplies, such as gloves and specimen cups. However, this cost estimate does not include: the machine’s electricity cost, the cost of daily assay control samples which are necessary to establish measurement parameters ($14-$80+/day), the costs of leasing the machine (about $2500 a year plus start-up costs), and the costs involved with lab staff training and salaries. These significant expenses would increase the seemingly reduced cost of EMIT testing in comparison to NicAlert. Additionally, NicAlert strips have a CPT code, which allows healthcare providers to bill insurers and government agencies to be reimbursed for the cost of their use. Another limitation when considering broader dissemination, is that like other methods that determine smoker status based on cotinine levels, NicAlert cannot be used when an individual is using nicotine replacement therapy. Overall, the limitations of the NicAlert strip can be balanced against the high specificity and sensitivity, the minimal training requirements, the lack of required equipment, and the ability to test cotinine level in almost any setting.

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NIH had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

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Table 1

Participant characteristics

Characteristics	**n = 50**
Demographics	
Age (years)	25.1 (.7)
Education (years)	12.1 (.3)
% Caucasian	92
% Married	16
% Private insurance	30
% First pregnancy	56
Weeks pregnant at intake	10.5 (.6)
Smoking Characteristics	
Cigarettes/day pre-pregnancy	20.3 (1.2)
Cigarettes/day in past 7 days	9.2 (1.0)
Age started smoking (years)	15.9 (.5)
Intake CO (ppm)	10.6 (.9)
Intake urinary cotinine (ng/ml)	988.7 (86.8)
% Living with other smoker(s)	82

Values represent mean (standard error) unless specified otherwise.

Table 2

Comparisons of performance between GC, EMIT and NicAlert

a)

NicAlert Scale	Expected cotinine value	# of samples present	# of samples expected	GC results of samples present in range
0	0–10 ng/mL	1	14	0 (n/a)
1	10–30 ng/mL	12	3	2.2 (1.8)
2	30–100 ng/mL	4	4	28.6 (18.0)
3	100–200 ng/mL	1	0	204 (n/a)
4	200–500 ng/mL	0	10	n/a
5	500–1000 ng/mL	8	8	268.1 (110.0)
6	>1000 ng/mL	22	9	923.2 (115.3)

b)

	True positive	True negative	False positive	False negative	Specificity (95% CI)	Sensitivity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)
NicAlert vs. GC	32	16	1	1	94% (69%–100%)	97% (82%–100%)	97% (82%–100%)	94% (69%–100%)
EMIT vs. GC	30	17	0	3	100% (77%–100%)	91% (75%–98%)	100% (85%–100%)	85% (61%–96%)

a) Semi-quantitative comparisions between NicAlert and GC. The two samples where observers did not agree on the NicAlert result have been removed. Expected cotinine value ranges are provided by the manufacturer. The number of samples expected is based on how many samples would be expected to fall into that range based on GC results. GC cotinine results are listed as mean (standard error).

b) Comparisons of accuracy in determining smoking status between EMIT and NicAlert. Note. CI, confidence interval.