

ORIGINAL ARTICLE

PILOT STUDY OF METHODS TO MEASURE SALIVA COTININE IN ALASKA NATIVE WOMEN DURING PREGNANCY

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ABSTRACT

Objectives. To evaluate NicAlert® immunoassay test strips and liquid chromatography atmospheric-pressure ionization tandem mass spectrometry (LC/MS/MS) for the measurement of saliva cotinine levels in pregnant Alaska Native women.

Study Design. Cross-sectional study.

Methods. Pregnant women completed a questionnaire about their tobacco use during pregnancy and that of others in the same household. Saliva samples were collected and tested using LC/MS/MS and NicAlert® immunoassay test strips.

Results. Seventy-one women participated; 18 reported using tobacco products during pregnancy. Sixteen women reported smoking within the last 7 days of the interview. Median cotinine concentrations in the women who reported smoking in the last 7 days were 94.5 ng/mL using LC/MS/MS (N=13) and 87.5 ng/mL using NicAlert® (N=11). Twenty-two women reported that they did not use tobacco and had no exposure to secondhand smoke. Median cotinine concentrations among those with no reported exposure to secondhand smoke were 0.0605 ng/mL using LC/MS/MS (N=18) and 4.38 ng/mL using NicAlert® (N=17).

Conclusion. NicAlert® strips can be used to quickly distinguish between users and nonusers of tobacco, but they are not adequately sensitive to detect and quantify lower concentrations of cotinine among nonsmokers with secondhand exposure. A more sensitive method such as LC/MS/MS is required for that purpose. (*Int J Circumpolar Health* 2007; 66(Suppl 1):29-38).

Keywords: Alaska Native, cotinine, pregnancy, saliva, smoking, secondhand smoke

INTRODUCTION

Tobacco exposure during pregnancy is a risk factor for poor birth outcomes (1). The goal of this pilot study was to assess methods for analyzing saliva samples in subsequent studies among Alaska Native people. This study provided background information for a subsequent 4-year study at the Alaska Native Medical Center.

Cotinine, a major metabolite of nicotine, has been used for about 25 years in studies of smoking and passive smoking (2-5). Cotinine can be used as a marker for active smoking and as an index for exposure to secondhand smoke (6). Cotinine is generally preferred over nicotine for such assessments because of its longer half-life. The estimated half-life of cotinine is 15-20 hours (4, 7-9) while the half-life of nicotine is 0.5-3 hours (7, 10, 11). Cotinine can be measured in serum, urine, or saliva – the half-life of cotinine in all three fluids is essentially the same (9). Previous studies suggest cutoff values of 10-15 ng/mL for serum or salivary cotinine to distinguish smokers from nonsmokers (5, 8, 9).

Nicotine and cotinine metabolism appear to differ by ethnic group and pregnancy status. The plasma cotinine levels of African American smokers are higher than the levels of white smokers when normalized to cigarettes per day (12-15). In addition, pregnancy itself may change the metabolism of nicotine and cotinine. Some research indicates that the clearance of nicotine and cotinine is significantly higher during pregnancy, and the half-life of cotinine is shorter (16, 17). Currently, no information exists about whether the levels of cotinine in pregnant Alaska Native/American Indian women differ from those of pregnant non-Native women.

MATERIAL AND METHODS

This study took place in the Women's Health Clinic at the Alaska Native Medical Center from June to August 2001 and was approved by the Alaska Area Institutional Review Board. All Alaska Native or American Indian pregnant women visiting the clinic were eligible to participate after providing informed consent. Each woman completed a questionnaire about her own tobacco use and that of others in the same household. In addition, they provided two simultaneous saliva samples using a Salivette® (Sarstedt, Newton, NC).

The Tobacco Exposure Biomarkers Laboratory at the Centers for Disease Control and Prevention in Atlanta, Georgia performed the analyses of cotinine. The saliva samples were tested first using the liquid chromatography atmospheric-pressure ionization tandem mass spectrometry (LC/MS/MS) method that has a level of detection of 0.015 ng/mL (18). Then, the saliva samples were tested with the NicAlert® immunoassay test strips followed by scanning and digital analysis to provide a more quantitative estimate of cotinine concentrations in the samples. This method has an estimated level of detection of 3 ng/mL (19).

The data from the questionnaires and the cotinine analyses were entered into a Microsoft Access database and imported into Statistical Analysis System (SAS) software for statistical analysis. Women using the nicotine patch, nicotine nasal sprays, nicotine inhalers or smoking cessation medications were excluded from the analysis because these could affect the cotinine results. We classified those women who indicated on the questionnaire that they smoked in the last 7 days as smokers. Women who indicated on the ques-

tionnaire that they were not users of tobacco were classified into three categories of second-hand smoke exposure based on their responses to the questionnaire:

1. Reported exposure from a husband or partner who smoked
2. Reported exposure from other sources including work areas, public work areas, smoking sections, and/or others smoking inside the home
3. No reported exposure to secondhand smoke

We used the Kruskal-Wallis test to assess whether the LC/MS/MS cotinine concentrations differed by reported tobacco exposure and whether the NicAlert® values differed by reported tobacco exposure. In addition, we used the Wilcoxon signed rank test to compare cotinine concentration distributions using the NicAlert® method and the LC/MS/MS method among the women who reported no tobacco use.

RESULTS

General population and sample description

Seventy-one women, with a mean age of 26 years volunteered for the study. Eighteen (26.9%) were in their first trimester of pregnancy, 24 (35.8%) were in their second trimester, and 25 (37.3%) were in their third trimester. Of the 71 women, two women did not provide saliva samples and one saliva sample did not have sufficient quantity for analysis. After the LC/MS/MS analysis, there were 61 samples with sufficient volume remaining to test using the NicAlert® method. One of those results remained inconclusive and was not included in the analysis.

Reported tobacco use from questionnaires

We tested several questions in this study to assess tobacco exposure. The majority (n=47, 66.2%) of the women had been smokers at one point in life, although the majority were not smoking when they found out they were pregnant (n=44, 62.0%). Twenty-two percent (16) of the women smoked a cigarette within the last 7 days and 77.5% (n=55) did not smoke in the last 7 days. Only one person reported using smokeless tobacco during her pregnancy and was excluded from the analysis (Table I).

Table I. Reported smoking status from the questionnaires (n=71).

Question	Number	%
Ever smoked cigarettes regularly (at least one cigarette a day for three months or longer)	47	66.2
Was smoking when found out for sure about this pregnancy	27	38.0
Had a cigarette, even a puff, in the last 30 days	19	26.8
Had a cigarette within the last 7 days	16	22.4
Used smokeless tobacco (chew, snuff, or iq'mik) during this pregnancy	1	1.4
Husband or partner who lives with woman now smokes tobacco	22	31
Husband or partner smoked inside the house during this pregnancy	8	11.3
Others (not including self or husband or partner) smoked cigarettes inside house during pregnancy	9	12.7
Employees frequently smoked in woman's work area or in public area while she was there	13	18.3
Regularly exposed to secondhand smoke at the job during this pregnancy	2	2.8

Comparing reported tobacco use from questionnaires with cotinine laboratory results

The median cotinine in the women who reported smoking cigarettes in the last 7 days was 94.5 ng/mL by LC/MS/MS (n=13) and 87.5 ng/mL by NicAlert® (n=11). Figures 1 and 2 display the cotinine concentrations obtained using LC/MS/MS and NicAlert® from women who reported smoking within the last 7 days. Of those women whose cotinine concentrations were less than 100 ng/mL, Figures 3 and 4 show the cotinine concentrations using LC/MS/MS and NicAlert® analyses according to the number of hours since they smoked their last cigarette. One value of 240 hours is not shown in this graph. The saliva cotinine results for this participant were 2.81 ng/mL using the LC/MS/MS method and 12.30 using the NicAlert® method. Figures 5

and 6 plot the average number of cigarettes smoked daily during the previous 7 days by the LC/MS/MS and NicAlert® methods (Figures 1-6).

Secondhand smoke exposure

Of those women whose husbands or partners smoked, the median cotinine concentrations were 0.105 ng/mL using the LC/MS/MS method (n=14) and 4.38 ng/mL using the NicAlert® method (n=14). Women exposed to secondhand smoke from other sources had median cotinine levels of 0.0470 ng/mL using the LC/MS/MS method (n=15) and 4.19 ng/mL using the NicAlert® method (n=16). Women with no reported secondhand smoke had median cotinine levels of 0.0605 ng/mL using the LC/MS/MS method (n=18) and 4.38 ng/mL using the NicAlert® method (n=17).

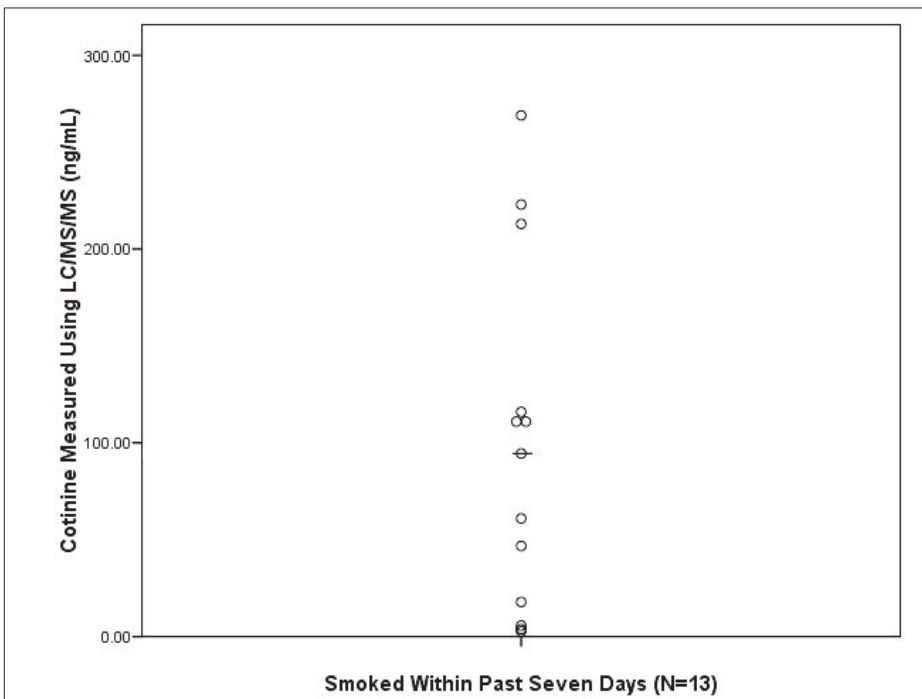


Figure 1. Saliva cotinine measured using LC/MS/MS (ng/mL) in women who smoked within the past 7 days. Horizontal line marks median value.

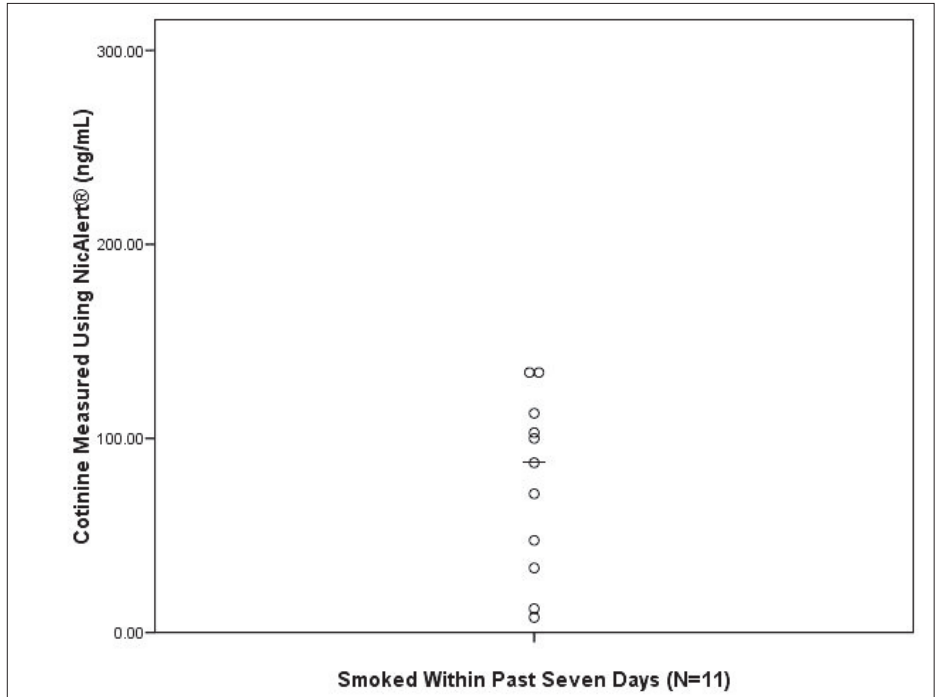


Figure 2. Saliva cotinine measured using NicAlert® (ng/mL) in women who smoked within the past 7 days. Horizontal line marks median value.

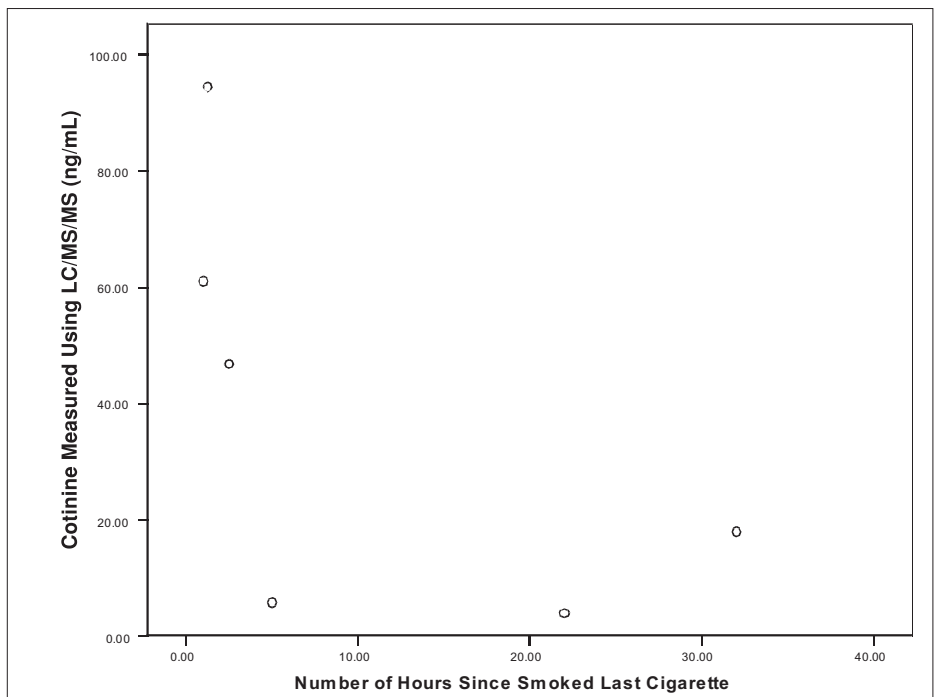


Figure 3. Relation between number of hours since smoked last cigarette and saliva cotinine measured using LC/MS/MS (ng/mL) for 6 women with cotinine < 100 ng/mL.

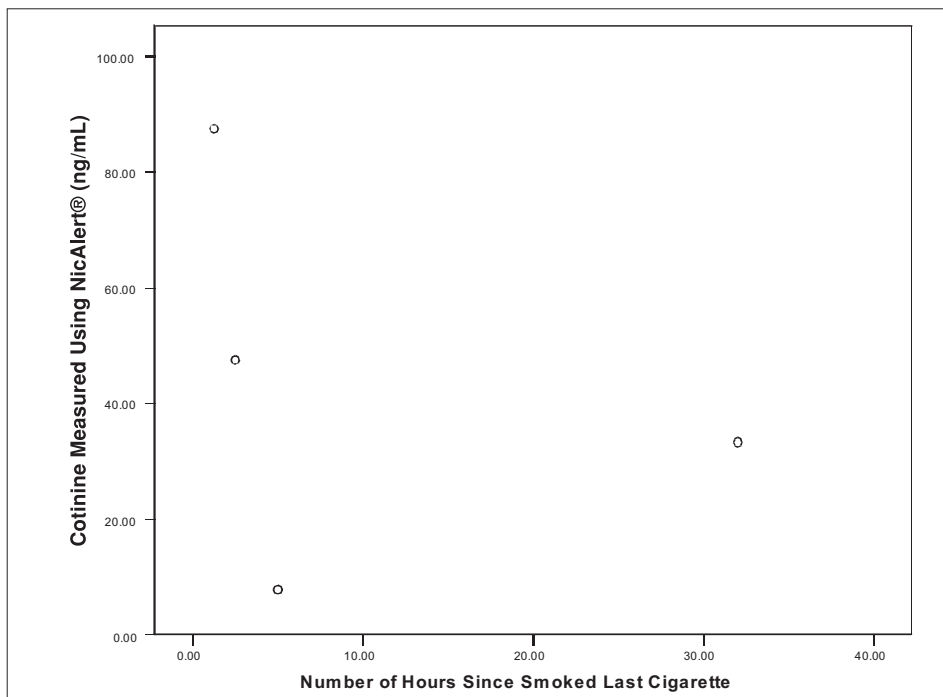


Figure 4. Relation between number of hours since smoked last cigarette and saliva cotinine measured using NicAlert® (ng/mL) for 4 women with cotinine < 100 ng/mL.

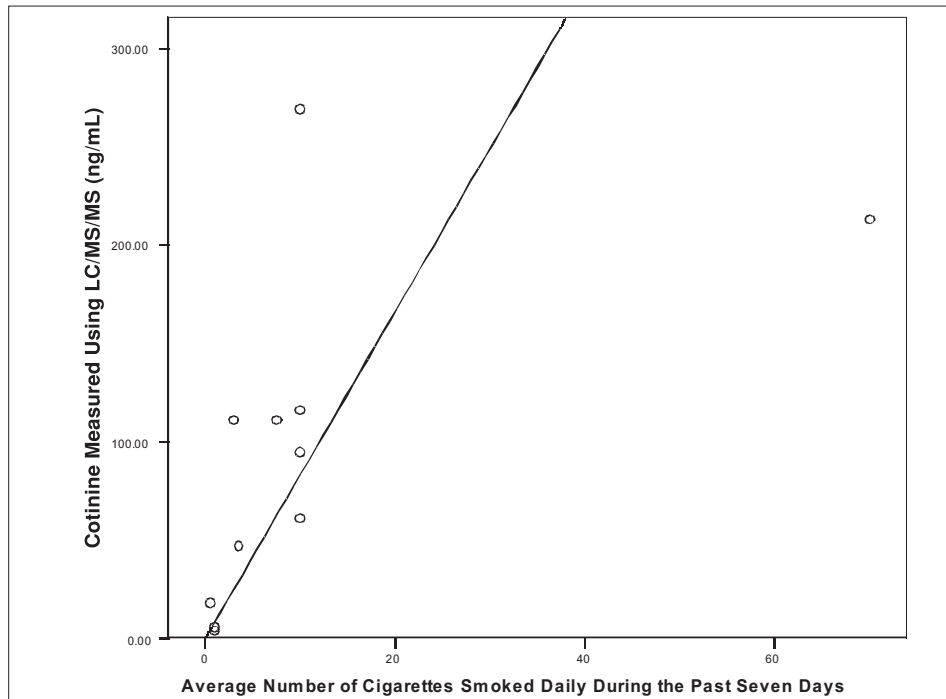


Figure 5. Relation between average number of cigarettes smoked daily during the past 7 days and saliva cotinine measured using LC/MS/MS (ng/mL).

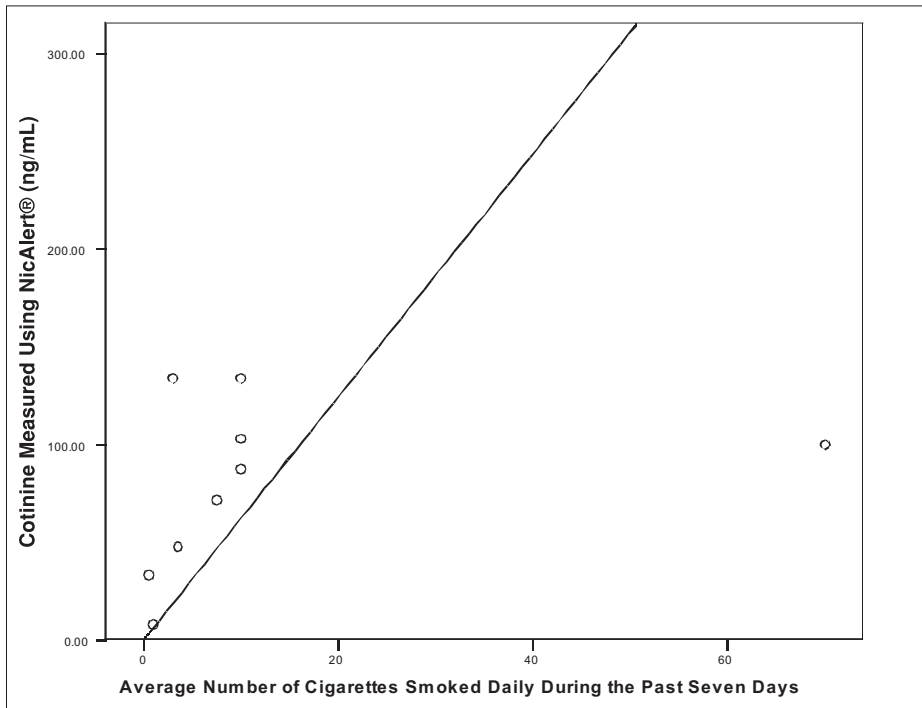


Figure 6. Relation between average number of cigarettes smoked daily during the past 7 days and saliva cotinine concentrations measured using NicAlert® (ng/mL).

Table II. Median saliva cotinine concentrations in pregnant Alaska Native women measured by liquid chromatography atmospheric-pressure ionization tandem mass spectrometry and NicAlert®. Range shown in parentheses.

Reported exposure	n	LC/MS/MS	n	NicAlert® (ng/mL)
No reported exposure to SHS	18	0.0605 (0.0210-0.446)	17	4.38 (2.85-6.46)
All other forms of reported SHS	15	0.0470 (0.020-0.335)	16	4.19 (2.30-5.72)
Husband or partner who smokes	14	0.105 (0.0380-2.09)	14	4.38 (3.70-8.25)
Smoker	13	94.5 (2.81-269)	11	87.5 (7.81-134)

SHS = secondhand smoke

LC/MS/MS = liquid chromatography atmospheric-pressure ionization tandem mass spectrometry ng/mL = nanograms of cotinine per milliliter of saliva

Using the Wilcoxon signed rank sum test, the NicAlert® method resulted in higher cotinine concentrations among those who reported no tobacco use than the LC/MS/MS method ($p < 0.0001$). We saw no statistically significant differences in the LC/MS/MS cotinine concentrations among women in the three categories of secondhand smoke exposure (Kruskal-Wallis test). Also, we saw no statistically significant differences in NicAlert®

cotinine concentrations among women in the three categories of secondhand smoke exposure (Table II).

Figures 7 and 8 show the LC/MS/MS and NicAlert® cotinine concentrations by the three categories of secondhand smoke exposure. The lines mark the median for each group. In Figure 7, the total number of non-detectable results is noted for each category. There were no non-detectable results using NicAlert®.

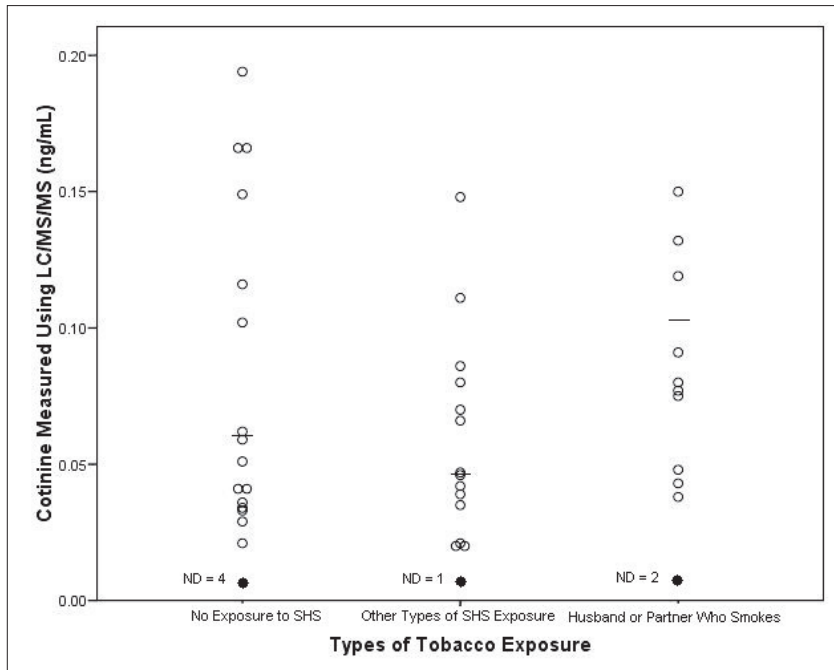


Figure 7. Saliva cotinine concentrations measured using LC/MS/MS (ng/mL) by categories of secondhand smoke (SHS) exposure. Horizontal lines mark median values.

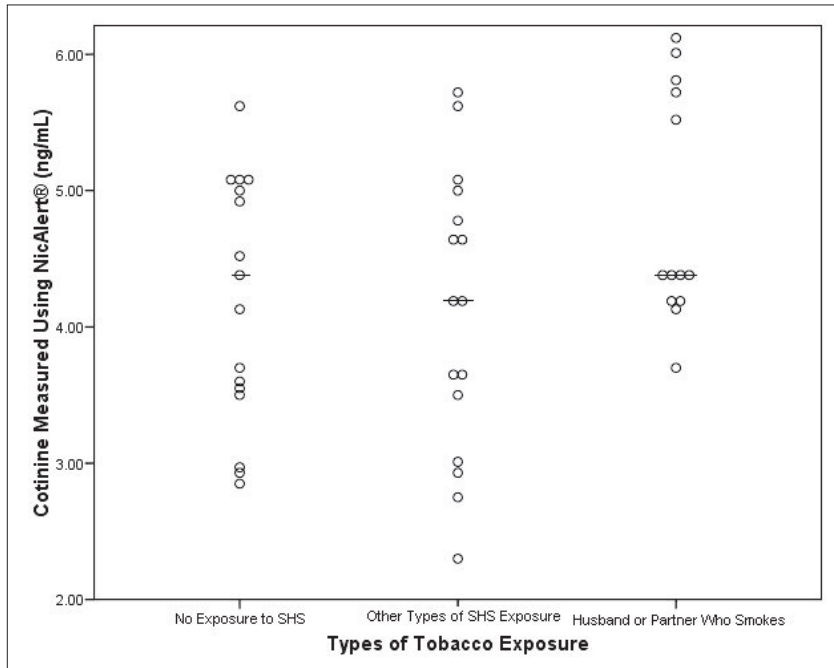


Figure 8. Saliva cotinine measured using NicAlert® (ng/mL) by categories of secondhand smoke (SHS) exposure. Horizontal lines mark median values.

DISCUSSION

We evaluated the use of NicAlert® for analysis of saliva cotinine by comparing its results with the data obtained by our primary method for cotinine analysis – LC/MS/MS. NicAlert® test strips provide rapid, semi-quantitative evaluation of smoking status without requiring elaborate and expensive laboratory facilities. Our results suggest that smoker or nonsmoker classification is feasible by using this simpler methodology with saliva samples. Using the NicAlert® test strips, however, resulted in erroneously high saliva cotinine concentrations among pregnant Alaska Native women who reported no use of tobacco compared to the LC/MS/MS method, which is considered the “gold standard”. The use of NicAlert® test strips resulted in misclassification of women’s secondhand smoke exposure. Women with no reported exposure to secondhand smoke were incorrectly classified by the NicAlert® test strips as exposed to secondhand smoke. This method does not have the necessary sensitivity to detect and quantify lower concentrations of cotinine among nonsmokers potentially exposed to secondhand smoke. A more sensitive method such as LC/MS/MS is required for that purpose.

Using the LC/MS/MS method, we were not able to detect statistically significantly higher cotinine concentrations in women who reported that a husband or partner smoked compared to women who reported that others smoked inside the home or that they were exposed to secondhand smoke in public work areas or smoking sections. However, this pilot study was not designed for that purpose. Because of the small sample size we may not have had adequate power to detect differences in saliva cotinine

concentrations among women in the 3 different categories of secondhand exposure status using LC/MS/MS. A study with a larger sample size would be needed to determine whether cotinine concentrations are higher in women with exposure to secondhand smoke from a husband or partner than in women exposed to other smokers inside the home or to smoke in public work areas or smoking sections.

In future studies among pregnant Alaska Native women, the use of the NicAlert® strips is recommended only in circumstances in which a rapid test is needed to distinguish between users and nonusers of tobacco. To detect and quantify lower concentrations of cotinine among nonsmokers potentially exposed to secondhand smoke a more sensitive method such as LC/MS/MS is recommended.

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