Original investigation

Fetal Exposure to Carcinogens With Tobacco Use in Pregnancy: Phase 1 MAW Study Findings

Christie A. Flanagan MPH¹, Kathryn R. Koller PhD¹, Abbie W. Wolfe MS¹,
Timothy K. Thomas MD¹, Neal L. Benowitz MD^{2,3}, Caroline C. Renner MPH¹,
Christine Hughes BS⁴, Dorothy K. Hatsukami PhD⁵, Carrie Bronars PhD⁴,
Neil J. Murphy MD⁶, Gretchen Day MPH¹, Paul A. Decker MS⁷,
Christi A. Patten PhD⁴

¹Clinical and Research Services, Division of Community Health Services, Alaska Native Tribal Health Consortium, Anchorage, AK; ²Center for Tobacco Control Research and Education, University of California, San Francisco, CA; ³Division of Clinical Pharmacology and Experimental Therapeutics, Medical Service, Departments of Medicine, and Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA; ⁴Department of Psychiatry and Psychology, Mayo Clinic, Rochester, MN; ⁵Department of Psychiatry, University of Minnesota, Minneapolis, MN; ⁶Southcentral Foundation, Department of Obstetrics and Gynecology, Alaska Native Medical Center, Anchorage, AK; ⁷Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester MN

Corresponding Author: Christie A. Flanagan, MPH, Clinical and Research Services, Division of Community Health Services, Alaska Native Tribal Health Consortium, 3900 Ambassador Drive, Suite 401, Anchorage, AK 99508-5922, USA. Telephone: 907-729-2948; Fax: 907-729-2924; E-mail: caflanagan@anthc.org

Abstract

Introduction: The high prevalence of smoking and smokeless tobacco (ST) use during pregnancy in Alaska Native (AN) women is concerning due to the detrimental effects of these products to the mother and the developing fetus. We sought to correlate maternal cotinine levels with fetal exposure to a tobacco-specific carcinogen to incorporate in a biomarker feedback intervention to motivate tobacco cessation during pregnancy.

Methods: Demographic and tobacco use data were collected from a convenience sample of pregnant AN smokers, ST users, and non-users. Maternal and neonatal urine were collected at delivery. Maternal urine cotinine and neonatal urine total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL, a tobacco-specific carcinogen) levels in smokers and ST users were analyzed and their correlations determined by Spearman correlation coefficients.

Results: During 2012–2014, we enrolled 64 non-users, 54 smokers, and 30 ST (20 homemade *iqmik*; 10 commercial ST) users ($n = 148$). Analyses of paired maternal-infant urine samples obtained for 36 smokers demonstrated a moderate to strong correlation ($r = 0.73$, $P < .001$) between maternal cotinine and infant NNAL levels. The correlation was not significant for 25 *iqmik* users ($r = 0.36$, $P = .17$) or 9 commercial ST users ($r = 0.60$, $P = .09$). No analysis was conducted for 55 non-users with cotinine and NNAL levels $<$ limits of quantification.

Conclusions: There is a moderate to strong correlation between maternal smoking and fetal exposure to the tobacco-specific carcinogen NNAL.

Implications: The correlation between maternal smoking and fetal carcinogen exposure may provide an education tool to help motivate smoking cessation among pregnant AN women. Further investigation is warranted to determine correlations between maternal commercial ST and *iqmik* use and neonatal NNAL.

Introduction

Despite a decrease in overall tobacco use in Alaska since the mid-1990s, more Alaskans die from tobacco exposure than from suicide, liver disease, vehicular crashes, and homicide combined.¹ Tobacco use prevalence continues to be higher among Alaskan adults compared to the US population. This high rate is driven primarily by the high cigarette and smokeless tobacco (ST) use prevalence (42% and 15%, respectively) among Alaska Native (AN) adults compared to non-Native Alaskan adults (19% and 4%, respectively), whose prevalence is similar to that of the United States over all (18% and 5%, respectively).

In 2010, 29.7% of AN mothers reported smoking during pregnancy compared to 9.3% of Alaska white mothers.² The prevalence of smoking among pregnant women in Alaska has significantly decreased during 1989–2010. However, there continues to be a high prevalence of tobacco use during pregnancy among AN women. The proportion of pregnant AN mothers who reported smoking during this time period has been consistently higher compared to pregnant Alaska white mothers. Similarly, while prenatal ST use in 1989–2010 decreased overall, the prevalence of ST use among AN women continued to be higher compared to Alaska white women.³ In 2010, prenatal ST use in AN women was 10.9% compared to 0.05% in Alaska white women. The elevated ST prevalence among AN women is driven primarily by the common use of *iqmik*, a home-made chewing tobacco made with tobacco leaves and ash.^{4,5} The ash is produced from burning willow bushes, alder, driftwood, or fungus (*punk*), which grows on birch trees.⁵

In addition to the high prevalence of tobacco use during pregnancy, AN women have lower spontaneous smoking quit rates and higher relapse rates postpartum than non-Native Alaskan women.⁶ Smoking in pregnancy increases the risk for low birth weight and pre-term birth and is associated with other negative outcomes for the infant.⁷ Using birth certificate data for 9175 live AN births, Murphy et al.⁸ showed that increasing numbers of cigarettes smoked per day during pregnancy resulted in a significant dose-wise decrease in mean infant birth weight. The mean birth weight of offspring of Yup'ik women who smoked greater than 10 cigarettes per day (CPD) was 3110g; 402g (11.3%) less than the mean birth weight of Yup'ik nonsmokers ($P < .05$). Less research has been conducted on the effects of ST use during pregnancy, but the available evidence suggests its use in pregnancy can have adverse effects on reproductive outcomes.^{6,9,10}

The high prevalence of prenatal tobacco use among AN women warrants exploration into interventions to improve cessation rates. The impetus for the current study was derived from a pilot study assessing the feasibility of a tobacco cessation intervention for AN women during pregnancy.¹¹ Sample size was too small to yield conclusive results, but women who did participate indicated a desire for objective information about specific harmful chemicals their infants might be exposed to, as motivation to quit.

The *Biomarker Feedback to Motivate Tobacco Cessation in Pregnant Alaska Native Women (MAW)* study is a 3-phase study designed to create and test a novel biomarker feedback intervention. In Phase 1, we aimed to establish the correlation between maternal tobacco use and fetal exposure to cancer-causing agents in pregnant AN women who use tobacco. We describe the methods, baseline participant characteristics, and urine biomarker results obtained from AN study participants in Phase 1 and summarize the relationships found between maternal urine cotinine (an established biomarker for nicotine use) and neonatal urine NNAL,

or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, a metabolite of NNK, the potent tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone,^{12,13} in AN women who used cigarettes or ST during pregnancy.

Materials and Methods

This study expanded upon a long-standing research partnership between the Mayo Clinic (Rochester, MN) and the Alaska Native Tribal Health Consortium (ANTHC, Anchorage, AK). Other collaborators included Sounthcentral Foundation (SCF, Anchorage, AK) and the University of California San Francisco (UCSF). ANTHC and SCF co-own and manage the Alaska Native Medical Center (ANMC), the inpatient facility on the Alaska Native Health Campus. SCF also manages primary care for American Indian and Alaska Native (AI/AN) residents of the Anchorage area and provides Obstetrics and Gynecology (OB/GYN) and Pediatric services for AI/AN women and children statewide. Study recruitment, enrollment, and data collection were conducted at these locations. Laboratory analyses of urine and plasma cotinine and carcinogens were conducted at the University of California San Francisco (UCSF).

An advisory committee was established and included members of the ANTHC study team, hospital and primary care staff, and members of the patient population. The purpose of the committee was to assist in developing study procedures, instruments, and materials prior to implementation as well as providing recommendations to ensure smooth study operations within and between outpatient and inpatient care areas.

The MAW study was approved by the Mayo Clinic, UCSF, and Alaska Area institutional review boards and the ANTHC and SCF Boards of Directors. To help protect participant privacy, a Certificate of Confidentiality was obtained from the Indian Health Service.¹⁴

Study Design

To examine biomarkers of exposure to nicotine and NNK we measured cotinine and NNAL concentrations in urine among maternal-infant pairs at delivery, with maternal tobacco use assessments conducted during pregnancy and at delivery. Cotinine, which has a longer half-life than nicotine, is a well-established biomarker for tobacco exposure and is easily measured in urine.¹² NNAL, the tobacco-specific carcinogenic metabolite of NNK, has an even longer half-life (10–16 days) and can also be measured in urine.^{12,13,15}

We sought to enroll a convenience sample of 165 pregnant women (55 smokers, 55 ST [commercial ST and *iqmik*] users, and 55 non- [tobacco] users) seeking prenatal care at SCF outpatient clinics. Non-users were included as a comparison group. ST use was defined as any type of tobacco used orally and not inhaled. The frequency and intensity of commercial ST and *iqmik* were measured by number of commercial ST-sized cans (about 1.2 oz.) used in the past 7 days.

Study inclusion criteria consisted of women of AN ethnicity who were: age 18 years or older, currently pregnant (any trimester) with a singleton pregnancy, and planned delivery at ANMC. An additional inclusion criterion for tobacco users was any tobacco use in the past 7 days. Women who had not used tobacco for at least 6 months were eligible for the non-user group. Women were not excluded from participating if they used more than one form of tobacco, but were assigned to either the smoker or ST user group based upon the tobacco type most frequently used because we did not anticipate a large number of multi-product users in this urban sample population. Women were excluded if they had used nicotine replacement

therapy or had participated in a behavioral or pharmacological tobacco cessation program within the previous 30 days.

Women were approached directly or referred by clinic staff. Interested women were screened for eligibility. Once informed consent was obtained, enrolled participants were assigned unique study identification (ID) numbers and completed the baseline assessments. This included an interviewer-administered questionnaire and urine collection.

Baseline Assessment

The baseline questionnaire was administered using a face-to-face interview format and included sociodemographic factors, length of gestation, number of children, spouse/partner tobacco use status, number of household tobacco users, and household restrictions on tobacco use. Possible responses to inquiry of restrictions on tobacco use inside the home included: no one is allowed to smoke anywhere, smoking is allowable in some places or at some times, or smoking is permitted anywhere anytime. For tobacco users, data on the amount and type of tobacco used were collected. Severity of nicotine dependence was measured in smokers using the Fagerström Test for Nicotine Dependence (FTND)¹⁶ and the Fagerström Tolerance Questionnaire for Smokeless Tobacco (FTQ-ST) was used for ST users.^{17,18} For non-users, lifetime tobacco use was assessed.

A baseline urine sample was collected to biochemically confirm self-reported tobacco use status. The specimen was immediately refrigerated at 4°C and within 48 hours an aliquot was removed and stored at -30°C. Study participants received a \$25 gift card once the baseline components were completed.

Delivery Assessment

Post-delivery specimen collection included maternal and infant urine samples. Infant urine was collected by placing cotton pads in the diaper, which were later compressed to obtain the infant urine, attempting to collect the earliest post-delivery void(s). Urine specimens were immediately refrigerated at 4°C and within 48 hours, aliquots were prepared and stored at -30°C.

Prior to discharge of the mother from the hospital, study staff administered a brief postpartum questionnaire to assess maternal tobacco use. For women who reported continued use, tobacco type and amount used in the past week were obtained. Participants received a second \$25 gift card after urine collection and completion of the postpartum assessment questionnaire.

Data/Specimen Management and Analysis

All assessment data were initially collected on paper forms and subsequently entered in an on-site study database. A quarterly database audit was performed by study coordinators on 100% of baseline and delivery assessments. Any discrepancies were documented and corrected. Cleaned, de-identified data were transferred by secure email to the Mayo Clinic and UCSF for analysis.

Frozen bio-specimens were batched and shipped overnight on dry-ice to the Clinical Pharmacology Tobacco Research Laboratory at UCSF for analysis. Concentrations of cotinine were determined by gas chromatography in maternal urine and liquid chromatography-tandem mass spectrometry in neonatal urine. The limits of quantification used to measure cotinine concentrations were 10 ng/mL in maternal urine and 0.05 ng/mL in neonatal urine.^{19,20} NNAL concentrations were determined by liquid chromatography-tandem mass spectrometry with a limit of quantification of 1.0 pg/mL.²¹ Maternal urine values were normalized to individual creatinine levels.

Statistical Analysis

All statistical analyses were conducted using SAS version 9.4. For descriptive data, the study population was stratified by tobacco use category (smoker, ST user, and non-user). Analysis of variance was used to assess differences between the means of normally distributed linear variables. Differences between tobacco use groups for categorical variables were determined using chi-square analysis. Maternal urine concentrations of cotinine were described using geometric means and 95% confidence intervals (CIs). Following convention, a value equal to the limits of quantification divided by the square root of two was used as a constant to maximize sample size when concentrations were below limits of quantification.²² The relationship between creatinine-normalized maternal cotinine and infant NNAL levels were analyzed using Spearman correlation coefficients, best suited for nonparametric data. *P*-values < .05 were considered statistically significant.

Results

During a 19-month recruitment period in 2012–2014, we screened 206 women who indicated interest in study participation. Of 172 eligible women, 148 (86%) enrolled: 64 nontobacco users, 54 smokers, and 30 ST users (20 *iqmik* and 10 commercial ST users). Reasons why some eligible women did not enroll included delivery before enrollment and loss of interest in study participation after study information was provided. Fewer than anticipated ST users were identified in the clinics during the enrollment period. Due to logistic issues, enrollment of ST users was terminated at 30 participants. Baseline characteristics were determined for ST users as a whole, rather than stratified by ST type. Sociodemographic and other characteristics of the enrolled study sample are presented in Table 1.

Among smokers, the mean number of CPD was 4.15 (*SD* = 3.18). Overall, ST users reported a mean of 0.8 commercial ST-size cans (1.2 oz per can) of ST per week. However, the mean number of equivalent size cans chewed per week by *iqmik* users was less than half (0.6, *SD* = 0.3) the number of cans per week reported by commercial ST users (1.2, *SD* = 1.1). Tobacco users (smokers, *P* = .02; ST users, *P* < .001) were likely to have had more children compared to non-users. Smokers, who reported living with more additional household smokers, also reported significantly more second hand smoke (SHS) exposure time than ST users and non-users (*P* < .001).

Biochemical confirmation of tobacco use status was based on criteria used in previous studies²³: a cotinine level of ≥50 ng/mL indicated active tobacco use and <50 ng/mL indicated non-use. Among the 64 non-users, 63 (98%) had cotinine levels <50 ng/mL. Among tobacco users, 50 of 54 (93%) women who reported smoking, 17 of 20 (85%) who reported *iqmik* use, and all 10 (100%) who reported commercial ST use were biochemically confirmed as active tobacco users (Table 2). Baseline assessments of self-reported number of cigarettes smoked per day were significantly correlated with maternal urine cotinine values (*r* = 0.47, *P* = .0003). Among ST users, self-report of the number of cans of ST used per week was correlated with maternal urine cotinine levels among commercial ST users (*r* = 0.74, *P* = .02), but not among *iqmik* users (*r* = -0.17, *P* = .47).

Of the 54 smokers, 36 (67%) maternal-infant paired urine samples were collected and analyzed for cotinine and NNAL content. There was a moderate to strong, positive correlation between maternal cotinine levels in our participants who smoked and their newborns' NNAL levels (*r* = 0.73; 95% CI: 0.53, 0.86; *P* < .001).

Table 1. Self-Reported Sample Characteristics

Characteristic	Non-users	Smokers	ST users	P	Total
At baseline— <i>n</i> (%)	64 (43)	54 (37)	30 (20)		148 (100)
Maternal age (in years)—mean (<i>SD</i>)	26.0 (5.1)	27.1 (5.0)	29.1 (5.7)	.24	27.1 (5.3)
Maternal age group (in years)— <i>n</i> (%)					
<24	22 (34)	17 (31)	5 (17)	.80	44 (30)
24–32	34 (53)	28 (52)	15 (50)		77 (52)
≥33	8 (13)	9 (17)	10 (33)		27 (18)
Urban vs. rural residence ^a — <i>n</i> (%)					
Urban	36 (56)	32 (59)	6 (20)	.74	74 (50)
Rural	28 (44)	22 (41)	24 (80)		74 (50)
Residence region— <i>n</i> (%)					
Southcentral	49 (77)	34 (63)	7 (23)	.11	90 (61)
Other ^b	15 (23)	20 (37)	23 (77)		58 (39)
# Other children—median (range)	1 (0–5)	2 (0–10)	3 (0–8)	.02	2 (0–10)
Spouse/partner is tobacco user— <i>n</i> (%)	35 (55)	42 (78)	19 (68)	.01	96 (66)
# Household smokers—mean (IQR)	0.5 (0–1)	1.5 (1–2)	0.8 (0–1)	.02	0.9 (0–1)
In home smoking ban— <i>n</i> (%)	57 (89)	43 (80)	27 (90)	.26	127 (86)
SHS exposure (in h/d)—mean (IQR)	1.4 (0–1)	5.8 (1–8)	1.9 (0–2)	<.01	3.1 (0–4)
FTND—median (range)	—	2 (0–7)	—	—	—
FTQ-ST—median (range)	—	—	1 (0–6)	—	—
Post-delivery					
Tobacco use at delivery— <i>n</i> (%)	67 (47)	48 (33)	29 (20)	—	144 (100)
Smokers # cigarettes/d—mean (<i>SD</i>)	—	4.7 (3.6)	—	—	—
ST users # cans/wk—mean (<i>SD</i>)	—	—	0.9 (1.06)	—	—

FTND = Fagerström Test for Nicotine Dependence, FTQ-ST = Fagerström Tolerance Questionnaire for Smokeless Tobacco; IQR = interquartile range; *n* = number; ST = smokeless tobacco; In-home smoking ban = no smoking permitted in home at any time; Tobacco use at delivery = self-reported use at time of delivery.

^aUrban = population ≥50 000; Rural = population <50 000; Rural includes urban clusters with populations 2500–49 999; US Census Bureau, www.census.gov/geo/reference/urban-rural.html, accessed February 11, 2016.

^bOther Alaska regional areas included Yukon-Kuskokwim, Arctic Slope, Bristol Bay, Interior, Norton Sound, and Northwest Arctic regions.

P < .05 = significant.

Table 2. Confirmation of Prenatal Self-Reported Tobacco Use Status by Urine Cotinine Level at Baseline

Tobacco use status (by self-report)	Cotinine cut-point	Cotinine IQR	Cotinine geometric mean (95% CI)
Non-users (<i>n</i> = 64)	<50 ng/mL	0–11	9.1 (8.1–10.2)
Smokers (<i>n</i> = 54)	≥50 ng/mL	77–910	307.5 (222.1–425.7)
<i>Iqmik</i> users (<i>n</i> = 20)	≥50 ng/mL	193–1645	587.3 (270.4–1275.7)
Commercial Chew Users (<i>n</i> = 10)	≥50 ng/mL	40–1536	987.6 (618.8–1576.4)

CI = confidence interval; IQR = interquartile range; *n* = number, ng/mL = nanograms per milliliter. Prenatal urine cotinine levels were creatinine-normalized.

Maternal-infant paired urine samples were obtained for 25 ST users. The correlation between maternal cotinine and newborn NNAL levels was not significant overall ($r = 0.09$; 95% CI: $-0.32, 0.47$; $P = .67$), nor when broken out by ST type: commercial ST users ($n = 9$; $r = 0.60$; 95% CI: $-0.11, 0.90$; $P = .088$; and *iqmik* users ($n = 16$; $r = 0.36$; 95% CI: $-0.16, 0.73$; $P = .17$). Similar results were obtained for the association of maternal cotinine and infant NNAL levels when the seven unconfirmed tobacco users were dropped: smokers ($r = 0.67$; 95% CI: $0.42, 0.82$; $P < .001$); all ST users ($r = -0.11$; 95% CI: $-0.52, 0.34$; $P = .63$); commercial ST users ($r = 0.61$; 95% CI: $-0.27, 0.93$; $P = .15$); and *iqmik* ($r = 0.05$; 95% CI: $-0.49, 0.57$; $P = .86$). Although the *iqmik* correlation reduces to 0.05, it is a weak correlation, as is the 0.36, and both are nonsignificant with these limited sample sizes. No correlation analysis was conducted for the 55 maternal-infant paired samples obtained from non-users with 98% cotinine levels <50 ng/mL.

Discussion

The present study is the first to examine exposure to the tobacco-specific carcinogen NNAL in infants of AN mothers who use tobacco during pregnancy. We found a moderate to strong positive correlation between maternal urine cotinine and infant urine NNAL with cigarette use. To date, only one study has previously investigated tobacco cessation interventions in pregnant AN women.¹¹ Anecdotal, AN women participating in this earlier pilot study conducted by Patten et al.¹¹ indicated that tangible evidence of fetal exposure to carcinogens as a result of tobacco use would help motivate cessation. We anticipate using this significant correlation to develop a population-specific intervention to motivate AN women who are pregnant to quit smoking.

We also found a positive correlation between maternal cotinine and infant NNAL in AN women who used ST products; however, the correlation was not significant. While the sample size was small

($n = 25$), important differences were noted between commercial and homemade (*iqmik*) that likely skewed the analysis of maternal cotinine and infant NNAL among ST users toward the null. Separately, the data suggest that fetal exposure to NNAL with maternal commercial ST use may be comparable or even greater than exposure related to maternal cigarette use. In contrast, with the 16 paired samples of women who used homemade ST (*iqmik*), the correlation between cotinine and NNAL was substantially weaker; infant NNAL levels remained consistently low regardless of the maternal cotinine level among *iqmik* users (see Figure 1 for linear fit). ST maternal cotinine results are limited by small sample size. However, our results are consistent with a prior study by Benowitz et al., which revealed plasma cotinine and urine nicotine levels in AN men and (nonpregnant) women were significantly higher among *iqmik* users compared to cigarette and other ST users.¹² Plasma cotinine and urine nicotine levels were comparable between groups.

Although statistical significance could not be determined in our study, clinical relevance cannot be overlooked; differences detected in NNAL exposure based on ST product type indicate that future analyses of commercial and homemade ST products should be conducted separately. Our findings also affirm previous observations of lower NNAL exposure in adult *iqmik* users compared to adult commercial ST users.¹² Importantly, while our results indicate low fetal exposure to NNAL with maternal *iqmik* use, they do not establish that *iqmik* is carcinogen-free or a safe alternative to cigarettes or commercial ST.

Our results also showed that self-report of tobacco use and urine cotinine level at baseline was correlated with self-reported amounts of tobacco use among cigarette smokers and commercial ST users. However, the average number of cigarettes smoked per day (4.15) was lower than amounts self-reported in other US population groups. Lower amounts of cigarettes smoked are consistent with findings from a previous study in which AN smokers reported an average of 7.8 CPD, whereas US smokers averaged 15 CPD, nearly double the average of AN smokers.¹² Despite this lower smoking rate, Benowitz et al.¹² found that the average plasma cotinine levels

in AN smokers were comparable to the average plasma cotinine levels among the overall US population. This finding, combined with studies indicating nicotine metabolism varies by race, sex, and genetic factors,²⁴ may indicate that not all smokers smoke with the same intensity (ie, smokers of fewer CPD smoke each cigarette more intensively than heavier smokers) or metabolize nicotine (and possibly other tobacco carcinogens) at the same rate. Research with AN people has shown that variation in the *CYP2A6* gene, which is the major pathway for metabolism of nicotine into cotinine, influences the rate at which nicotine is metabolized, which in turn, influences tobacco consumption.²⁵

In terms of ST use, the average number of cans used per week in our sample was 0.6 among *iqmik* users and 1.2 among commercial ST users. Similarly, Benowitz et al.¹² found that *iqmik* users averaged fewer (1.2) cans per week compared to commercial ST users (1.6 cans per week). This difference may be explained by the greater amount of nicotine delivered by *iqmik* compared to commercially prepared ST products. This homemade ST, a mixture of tobacco leaves and fungus ash, results in higher nicotine exposure than use of other tobacco products. The addition of ash raises the pH of the tobacco and increases the amount of free (unionized) nicotine available for absorption.^{5,26} Therefore, self-report of CPD smoked or cans per week of ST used may not adequately reflect individual nicotine or other chemical exposure. The correlation between maternal cotinine and infant NNAL, despite reduced tobacco consumption, legitimizes concerns over fetal exposure to carcinogens. Cessation efforts must focus on getting pregnant AN women to quit tobacco use completely, as opposed to reducing or “cutting back” the amount of tobacco used.

The small sample size for ST users is a limitation of the study. The majority of women in Alaska who reported prenatal ST use (42.1%) resided in the western Alaska region.⁴ Women with higher risk pregnancies in this region have traditionally been referred to ANMC for prenatal care and delivery. A relatively recent increase in obstetrics care capacity at one of the western regional hospitals has reduced the number of women requiring referral to ANMC, which thereby

Figure 1. Linear fit for correlation between creatinine-normalized urine cotinine levels in mothers who used tobacco in pregnancy and infant urine 4-(methylnitroamino)-1-(3-pyridyl)-1-butanol (NNAL) levels.

reduced the number of prenatal ST users attending the SCF clinics. Despite this limitation, our results suggest that NNAL may be a good biomarker for estimating fetal exposure to carcinogens among commercial ST users, but a larger sample size is necessary to determine this definitively. Given the lack of correlation between cotinine and NNAL with *iqmik* use, assessment of exposure to other carcinogens found in *iqmik*,²⁷ particularly heavy metals, is currently underway. Another limitation is the low number of mother-infant paired urine collections. In some cases, clinical contingencies precluded collection of study materials. Additionally, not all neonatal first-voided specimens could be collected, as urine required for clinical purposes assumed priority. Overall, convenience sampling and the exclusion of women who recently quit tobacco and/or were using nicotine replacement therapy limits generalizability beyond AN women who currently smoke or use ST while pregnant. Studies with larger sample sizes are needed to confirm findings in this AN population and other populations.

Smoking not only affects the health of the mother and mean birth weights,⁸ but also the viability of the fetus and the propensity for tobacco dependence among offspring.²⁸ In our study, we demonstrated a correlation between maternal cotinine levels and newborn levels of carcinogenic NNAL in women who smoked during pregnancy. Our results also indicate that maternal commercial ST use may produce similar exposure. In a population with high tobacco use prevalence, effective methods to help AN women stop smoking during pregnancy are critical. Although tobacco cessation programs exist in the Tribal health system, they do not specifically focus on prenatal patients and are underutilized by pregnant women. In Phase 2 we will incorporate our findings in a biomarker feedback intervention specifically designed to help motivate pregnant AN women quit tobacco use in pregnancy and will assess the effectiveness of this intervention in Phase 3.

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Declaration of Interests

NLB is a consultant to several pharmaceutical companies that market medications to aid smoking cessation and has served as a paid expert witness in litigation against tobacco companies. The other authors have no conflicts to disclose.

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