Prenatal Tobacco Exposure, Biomarkers for Tobacco in Meconium, and Neonatal Growth Outcomes

Sarah K. Himes, BS[1], Laura R. Stroud, PhD[2], Karl B. Scheidweiler, PhD[1], Raymond S. Niaura, PhD[3], and Marilyn A. Huestis, PhD[1]

Objective To assess relationships between marker concentrations of tobacco in meconium and weekly self-reported maternal cigarette consumption, and prediction of neonatal growth outcomes.

Study design Pregnant mothers (n = 119) from a longitudinal maternal smoking and infant neurobehavioral study (Behavior and Mood in Babies and Mothers [BAM BAM]) provided daily tobacco smoking histories. Nicotine, cotinine, and *trans*-3′-hydroxycotinine concentrations were quantified in 111 neonatal meconium specimens by liquid chromatography-tandem mass spectrometry.

Results Median self-reported third trimester smoking was 5.9 cigarettes per day among smokers. Meconium samples from infants born to non-smokers (n = 42) were negative for tobacco markers, while specimens from self-reported smokers (n = 41) were positive for (median, range) nicotine (50.1 ng/g, 3.9-294), cotinine (73.9 ng/g, 6.4-329), and *trans*-3′-hydroxycotinine (124.5 ng/g, 10.2-478). Quitters (n = 28) self-reported stopping smoking at gestational weeks 2-39. Four meconium specimens from quitters were positive for tobacco biomarkers. Reduced birth weight, length, and head circumference significantly correlated with presence of meconium markers but not with individual or total marker concentrations. Among quitters and smokers, reduced infant birth weight, head circumference, and gestational age correlated with total and average daily cigarette consumption in the second and third trimesters.

Conclusion Smoking cessation or reduction during pregnancy improved neonatal outcomes. The window of detection for tobacco in meconium appears to be the third trimester; however, low exposure in this trimester failed to be detected. These results will aid physicians in educating women who are pregnant or thinking about becoming pregnant on the negative consequences of smoking during pregnancy. In addition, infants at risk can be identified at birth to assist early intervention efforts. *(J Pediatr 2013;162:970-5).*

D espite strong public health warnings, 16.3% of women smoke tobacco throughout pregnancy.[1] In utero tobacco exposure is associated with decreased fetal growth, increased infant irritability and hypertonicity, alterations in brain structure or function, and increased risk of sudden infant death syndrome.[2-5] Gestational tobacco exposure also is associated with deficits in cognitive function, and increased risk for behavioral disorders, attention deficit/hyperactivity disorder, and tobacco dependence later in life.[6-9]

Biological monitoring of maternal and neonatal specimens is a more objective measure of duration and intensity of in utero tobacco exposure than maternal self-report, as these accounts are subject to recall bias and social stigmas.[10,11] Testing of meconium, which begins to form at the 13th gestational week,[12] is an advantageous neonatal matrix because of the ease of collection, adequate specimen volume, and long window of drug detection[13]; however, contribution from secondhand/environmental exposure is still unclear.[14,15] In adults, nicotine is oxidized to cotinine and further metabolized to *trans*-3′-hydroxycotinine (OHCOT), primarily by CYP2A13 and CYP2A6.[16]

The aims of this study were to assess relationships between marker concentrations of tobacco in meconium and daily self-reported maternal cigarette consumption, and prediction of neonatal growth outcomes. There are limited studies of utilizing marker concentrations of tobacco in meconium, particularly those of nicotine and OHCOT, to validate maternal self-report and predict neonatal outcomes. A better understanding of the relationships between tobacco markers in meconium, maternal smoking, and neonatal outcomes is needed, as these data could identify children at risk and strengthen smoking cessation interventions during and following pregnancy.

LOQ	Limits of quantification
OHCOT	*Trans*-3′-hydroxycotinine
SES	Socioeconomic status
THC	Δ^9-tetrahydrocannabinol
THCCOOH	11-nor-9-carboxy-THC

Methods

Women were enrolled in the Behavior and Mood in Babies and Mothers [BAM BAM; PI: L.S.] study, a longitudinal study assessing effects of gestational tobacco exposure on infant neurobehavior. Recruiting was designed to oversample smokers to enroll smoking and non-smoking maternal cohorts. The study was approved by the Institutional Review Board of Women and Infants' Hospital (Providence, Rhode Island), and mothers provided written informed consent. Pregnant women ages 18-40 years were enrolled during the third trimester and gave detailed daily tobacco intake information throughout pregnancy. Initial study visits occurred at ≥28 weeks, with 1-2 follow-up interviews at 34-38 weeks, and a perinatal interview at delivery. Self-reported cocaine or other illicit drug use other than cannabis was an exclusion. At each visit, trained interviewers recorded cigarettes smoked each day of pregnancy using the timeline follow-back methodology. Total and mean cigarettes smoked per day during each gestational week were calculated. Secondhand smoke exposure was assessed weekly with questions on mean weekly hours of exposure in and outside the home.[17] Oral fluid specimens were collected by passive drool during the third trimester and assayed for cotinine to provide additional biochemical verification of maternal smoking status.

Meconium specimens were collected within 72 hours of delivery by combining samples from multiple soiled diapers until appearance of milk stool. Meconium from multiple diapers was collected in a single polypropylene container and shipped frozen to the National Institute on Drug Abuse for analysis. Cotinine, nicotine, and OHCOT concentrations were quantified in meconium by a validated liquid chromatography-tandem mass spectrometry method.[18] Liquid chromatography-tandem mass spectrometry limits of quantification (LOQ) were 2.5 ng/g nicotine, 1.0 ng/g cotinine, and 5.0 ng/g OHCOT. This method also quantified opioids, methamphetamine, cocaine, and several metabolites.[18] A separate 2-dimensional gas chromatography mass spectrometry method quantified cannabinoids, Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-THC, 8β,11-dihydroxy-THC, cannabinol, and 11-nor-9-carboxy-THC (THCCOOH) in meconium.[19] Oral fluid specimens were analyzed for cotinine by Salimetrics (Salimetrics LLC, State College, Pennsylvania) with an enzyme-linked immunosorbent assay with a LOQ of 0.15 ng/mL.

Statistical calculations were performed with GraphPad Prism 5 (GraphPad Software, Inc, La Jolla, California) and SPSS 16.0 for Windows (SPSS Inc, Chicago, Illinois). Normality was assessed with visual inspection of box plots and Kolmogorov–Smirnov tests. Nonparametric t tests evaluated differences between 2 groups and nonparametric one-way ANOVA determined group differences between 3 or more groups. Pearson and Spearman correlations evaluated associations between normally and non-normally distributed variables, respectively. ANCOVA and multiple regression models were employed to adjust for covariates. Statistical probability $P < .05$ was considered statistically significant for all comparisons.

Results

Criteria for study inclusion were met by 119 women: 46 (38.7%) denied smoking during pregnancy ("nonsmokers"), 45 (37.8%) smoked throughout pregnancy ("smokers"), and 28 (23.5%) reported quitting prior to delivery ("quitters"). Meconium specimens from 111 infants were present in sufficient quantity for analysis.

Maternal and infant characteristics for each group are summarized in Table I. Significant group differences were seen for group percent with low socioeconomic status (SES) (based on a score of 4 or 5 on the Hollingshead Index, which assesses SES by evaluating parental social position, including occupations and education of both parents),[20] and average secondhand smoke exposure >1 h/d throughout pregnancy (Table I). Infant birth weight, length, and head circumference were significantly different between nonsmokers, quitters, and smokers, with the smokers' infants having the lowest weight and shortest length and head circumference (Table I). Total trimester cigarettes smoked and average daily cigarettes smoked for self-reported quitters and smokers are shown in Table II. Mann–Whitney t tests indicated that all variables described in Table II were significantly different between quitters and smokers ($P < .0001$).

Meconium samples were negative (<LOQ) for all 3 tobacco markers from all self-reported non-smokers and positive for all self-reported smokers (women who continued to smoke until delivery) (Table II). Quitters (n = 28) self-reported stopping smoking in gestational weeks 2-39. Only 4 meconium specimens from infants born to quitters were positive for tobacco biomarkers. These mothers reportedly quit smoking at gestational weeks 9, 12, 16, and 39. Median concentrations of meconium in the 4 positive quitter specimens (nicotine 4.3 ng/g, cotinine 4.4 ng/g, and OHCOT 20.6 ng/g) were 5-15 times lower than those in smokers' meconium (Table II). Quitters also smoked 2-9 times fewer cigarettes per day throughout pregnancy than smokers (Table II).

Oral fluid cotinine measurements verified or refuted maternal self-reported smoking behavior. Self-reported quitters with negative meconium all had third trimester oral fluid specimens that verified maternal self-report. In these women, oral fluid cotinine was <6.5 ng/mL, except for a single oral fluid sample from a week-33 quitter taken 10 weeks prior to delivery with cotinine present at 18.7 ng/mL; these results are consistent with maternal self-report.

Self-reported quitters with positive meconium reportedly quit smoking during gestational weeks 9, 12, 16, and 39; however, third trimester oral fluid specimens were positive for week 9 and 16 quitters, suggesting continued smoking