Brief report

Opioid Use and Rate of Nicotine Metabolism Among Pregnant Smokers

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Abstract

Introduction: Smokers who use opioids smoke more cigarettes per day (CPD) than non-opioid users, which could be due to the effects of opioids on nicotine metabolism. Moreover, nicotine metabolism increases during pregnancy, potentially making quitting more difficult for pregnant smokers. We examined nicotine metabolism and its association with opioid use (OU) and CPD in pregnant smokers.

Methods: We recruited pregnant women who smoked at least 5 CPD for a clinical trial of smoking cessation. Plasma nicotine metabolite ratio (NMR; *trans*-3'-hydroxycotinine (3HC)/cotinine)—a biomarker of nicotine metabolism—OU (involving methadone, buprenorphine, fentanyl, oxycodone, or tramadol), and CPD were assessed at baseline. We used linear regression to examine the associations between log-transformed NMR, OU, and CPD, adjusting for race/ethnicity and menthol smoking.

Results: Among 129 pregnant smokers, 25 (19%) were opioid users; most were maintained on methadone ($n = 14$). Compared to non-OU smokers, OU smokers had higher median CPD (10.0 vs. 7.0, $p = .0007$), serum 3HC (81.0 vs. 42.0 ng/mL, $p = .0001$), and NMR (0.63 vs. 0.43, $p < .0001$). In addition, methadone-maintained smokers had a higher median NMR than non-OU smokers (0.66 vs. 0.43, $p = .0004$). Adjusting for covariates, log-transformed NMR was greater in OU smokers ($p = .012$), specifically methadone-maintained smokers ($p = .024$), than non-OU smokers.

Conclusions: Our preliminary results show that OU is associated with a higher NMR in pregnant smokers. A larger study sample is needed to replicate this finding, examine potential mechanisms, and determine its clinical significance.

Implications: Among pregnant smokers, we observed that nicotine metabolism was significantly faster among opioid users—the majority of whom were on methadone maintenance—compared to nonusers, which could have implications for smoking cessation. Further studies are needed to replicate this finding, evaluate potential mechanisms, and determine its clinical significance.

Introduction

Cigarette smoking during pregnancy is one of the most important modifiable causes of poor pregnancy outcomes in the United States.¹ Smoking among pregnant women is associated with low birth

weight, premature birth, placental abruption, birth defects, and infant mortality.¹ Smoking cessation can significantly improve babies' outcomes.¹ Unfortunately, about 75% of women who smoked prior to pregnancy continue during pregnancy.² In addition, nicotine

replacement therapy, which is a standard treatment for smoking cessation, is less effective in this population¹ likely due to increased nicotine metabolism during pregnancy.³

Chronic use of opioids (eg, methadone and pain medications such as oxycodone) may present further challenges for smoking cessation during pregnancy. Opioid-dependent smokers exhibit greater nicotine dependence than non-opioid-dependent smokers.⁴ Heavy smoking is associated with higher rates of neonatal abstinence syndrome and longer hospitalization among pregnant women in treatment for opioid use (OU),⁵ illustrating the need to understand the relationship between chronic OU and smoking during pregnancy.

Differences in the rate at which smokers metabolize nicotine may help to explain these findings. Most nicotine is metabolized to cotinine by the enzyme *CYP2A6*, and *CYP2B6* is a minor pathway of metabolism. Next, cotinine is converted to *trans*-3'-hydroxycotinine (3HC) exclusively by *CYP2A6*.³ The 3HC/cotinine ratio—the nicotine metabolite ratio (NMR)—in plasma is a good marker of *CYP2A6* activity. Smokers who metabolize nicotine more rapidly smoke more CPD.⁶ Women are, on average, faster metabolizers than men,⁷ and nicotine metabolism accelerates during pregnancy,³ potentially making smoking cessation more difficult for pregnant smokers.

Smokers who are chronic opioid users may metabolize nicotine more rapidly than nonusers. One possible reason for this is that opioids activate nuclear hormone receptors, which facilitate the upregulation of genes known to also be involved in nicotine metabolism.⁶ Methadone activates the pregnane X receptor (PXR) and constitutive androstane receptors,⁸ resulting in increased transcription of *CYP2B6*, *CYP3A4*, *UGT1A1*, and *MDR1*. Nicotine is metabolized in part by *CYP2B6*,⁶ the expression of which is induced by methadone and estrogen.^{8,9} Although *CYP2B6* is a minor pathway for nicotine metabolism, its induction could alter nicotine metabolism. Further, although it is unknown whether activation of PXR and constitutive androstane receptors by methadone increases *CYP2A6* mRNA expression,⁸ it is plausible, as these nuclear receptors are known to regulate nicotine metabolism.⁶ OU and pregnancy are also associated with hypercholesterolemia,^{10,11} which would be expected to activate PXR for both cholesterol and hormone breakdown and regulation¹² and could indirectly influence *CYP2A6* expression. Finally, morphine treatment increased cholesterol accumulation and bile acid dysregulation in the liver in experimental animals,¹³ which could increase the expression of *AKR1D1*, another gene postulated to be a regulator of nicotine metabolism.⁶

The purpose of this pilot study was to explore whether, during pregnancy, smokers who use opioids have a greater NMR than those who do not use opioids. Given the clinical association between OU and heavier smoking, we hypothesized that pregnant smokers who use opioids metabolize nicotine more rapidly—as evidenced by an increased NMR—than those who do not use opioids.

Methods

Participants

This study was part of a randomized controlled trial of the efficacy of a nicotine versus placebo inhaler for smoking cessation during pregnancy ($N = 137$). Detailed study methods are available at ClinicalTrials.gov (NCT01656733). Pregnant women were eligible if they (1) smoked at least 5 cigarettes per day (CPD) on average, (2) had tried to quit smoking during this pregnancy and currently wanted to quit, (3) were at 13–26 weeks of gestation, (4) were at least 16 years old, (5) were able to speak English or Spanish, (6) intended to carry

their pregnancy to term, and (7) lived in a stable residence. We excluded individuals with (1) current drug abuse or dependence (other than nicotine and licit OU) or had reported use of any illicit drug since learning of pregnancy (with the exception of marijuana), (2) twins or other multiple gestation, (3) an unstable psychiatric or medical problem, or (4) a known congenital abnormality. Institutional review boards at UConn Health (Farmington, Connecticut), Hartford Hospital (Hartford, Connecticut), and Baystate Medical Center (Springfield, Massachusetts) approved the study. The study was conducted from August 2012 through January 2017.

The current analyses included 129 pregnant smokers with complete biomarker data at baseline; we excluded seven participants with no serum for laboratory analysis, and one participant with cotinine and 3HC levels below the level of quantification.

Procedure and Measures

Baseline demographic variables included age, gestational age (in weeks), and race/ethnicity. We obtained a medication history, and OU was considered positive if the participant was in treatment for OU (receiving methadone or buprenorphine) or reported regularly taking fentanyl, oxycodone, or tramadol. Duration of OU was estimated from medical records and participant interviews available for 18 participants, and estimated only for prescription medications within these health systems. Preference for menthol cigarettes was assessed at screening. At baseline, the average number of cigarettes smoked per day in the past 7 days was assessed by self-report, and the Fagerström Test for Cigarette Dependence was administered.

Analytical Chemistry

Serum samples were analyzed for concentrations of cotinine and 3HC using high performance liquid chromatography with tandem mass spectrometry at Yale University, as previously described.¹⁴

Data Analysis

NMR was calculated as the ratio of 3HC/cotinine.³ In bivariate analyses, we tested differences in baseline characteristics by OU using Wilcoxon rank-sum (Mann–Whitney), Fisher's exact, and chi-square tests. Because NMR was not normally distributed, we used log-transformed NMR in the regression model, as has been done previously.¹⁵ We conducted multivariable linear regression to determine the associations between log-transformed NMR and OU, CPD, menthol smoking, and race/ethnicity. Owing to the small sample of OU smokers, we prioritized the aforementioned factors, which differed by OU status and were previously shown to be important predictors of NMR.⁶ In addition, to examine the associations between specific types of opioids and log-transformed NMR, we conducted a multivariable linear regression of log-transformed NMR in which the main predictor was a four-level variable for opioid type (nonuse of opioids [reference group], methadone maintenance, buprenorphine use, and use of other opioids), adjusting for the same covariates. We reported regression b coefficients (not standardized β s) and considered associations significant at $\alpha < 0.05$. All analyses were performed using Stata version 15 (StataCorp, College Station, TX).

Results

Sample Characteristics

Baseline demographic and smoking characteristics of the sample of pregnant smokers ($N = 129$) are shown in Table 1. Most of the continuous variables in Table 1 were not normally distributed (with the

Table 1. Characteristics and Nicotine Metabolites Among Pregnant Smokers by Opioid Use Status

	Nonuser (<i>n</i> = 104)	User (<i>n</i> = 25)	<i>p</i> value
Age in years, median (25th, 75th percentile)	27.0 (22.5, 31.0)	29.0 (26.0, 34.0)	.0988 ^a
Race/ethnicity, <i>n</i> (%)			<.0001 ^b
White	30 (28.9)	21 (84.0)	
Black	20 (19.2)	0 (0)	
Hispanic/Latina	52 (50.0)	3 (12.0)	
Other/refused	2 (1.9)	1 (4.0)	
Gestational age in weeks, median (25th, 75th percentile)	16.8 (13.6, 19.6)	17.1 (14.1, 21.6)	.6784 ^a
Cigarettes per day, median (25th, 75th percentile)	7.0 (5.0, 10.0)	10.0 (8.0, 15.0)	.0007 ^a
Menthol smokers, <i>n</i> (%)	89 (85.6)	17 (68.0)	.039 ^c
Fagerström Test for Cigarette Dependence score, median (25th, 75th percentile)	4.0 (3.0, 6.0)	5.0 (4.0, 6.0)	.0462 ^a
Serum cotinine (ng/mL), median (25th, 75th percentile)	107.5 (64.0, 145.7)	136.0 (93.0, 174.0)	.1206 ^a
Serum 3HC (ng/mL), median (25th, 75th percentile)	42.0 (26.0, 64.0)	81.0 (52.0, 113.0)	.0001 ^a
Nicotine metabolism ratio, median (25th, 75th percentile)	0.43 (0.32, 0.56)	0.63 (0.49, 0.83)	<.0001 ^a
Type of opioid, <i>n</i> (%)			
Methadone	—	14 (56.0)	—
Buprenorphine	—	8 (32.0)	—
Other ^d	—	3 (12.0)	—
Duration of opioid use in months, median (25th, 75th percentile) (<i>n</i> = 18) ^e	—	9.0 (2.0, 24.0)	—
Duration of methadone use in months, median (25th, 75th percentile)	—	16.0 (4.0, 48.0)	—
Duration of buprenorphine use in months, median (25th, 75th percentile)	—	3.0 (2.0, 4.0)	—
Duration of “other” opioid use in months, median (25th, 75th percentile)	—	10.0 (2.0, 18.0)	—

^a*p* values calculated using Wilcoxon–Mann–Whitney tests.

^b*p* values calculated using Fisher’s exact tests.

^c*p* values calculated using chi-square tests.

^dIncludes fentanyl (*n* = 1), oxycodone (*n* = 1), and tramadol (*n* = 1).

^eDuration of prescription opioid use was estimated from medical charts and patient interviews. Information was missing for seven participants (three methadone, three buprenorphine, and one tramadol).

exception of age) so we displayed medians and interquartile ranges (IQRs) to provide the best characterization of the data. The median (IQR) ages were 29.0 (26.0, 34.0) years among OU smokers and 27.0 (22.5, 31.0) years among non-OU smokers. Half of non-OU smokers were Hispanic or Latina (50.0%), and most OU smokers were non-Hispanic white (84.0%, *p* < .0001). Median (IQR) CPD was higher in OU smokers [10.0 (8.0, 15.0) vs. 7.0 (5.0, 10.0)], who were less likely to smoke menthols (68.0% vs. 85.6%, *p* = .039), than non-OU smokers. Most OU smokers used methadone (56.0%), followed by buprenorphine (32.0%). The median (IQR) duration of OU was about 9 (2.0, 24.0) months among all opioid users, and 16 (4.0, 48.0) months among methadone users.

Biomarkers of Nicotine Metabolites

Serum cotinine did not differ by OU status (Table 1). However, OU smokers had higher median (IQR) levels of serum 3HC [81.0 (52.0, 113.0)] than non-OU smokers [42.0 (26.0, 64.0), *p* = .0001]. Thus, OU smokers had a higher median (IQR) NMR [0.63 (0.49, 0.83)] than non-OU smokers [0.43 (0.32, 0.56), *p* < .0001]. Median (IQR) NMR was 0.66 (0.49, 0.98) among methadone users, 0.63 (0.63, 0.96) among “other” users, and 0.55 (0.42, 0.74) among buprenorphine users (data not shown). Median NMR was higher in methadone-maintained smokers than non-OU smokers (*p* = .0004).

Predictors of Nicotine Metabolism Ratio

The results of the linear regression model of log-transformed NMR adjusted for menthol smoking and race/ethnicity are presented in Table 2. OU smokers had a faster rate of nicotine metabolism than non-OU smokers (*b* = 0.29, SE = 0.11, *p* = .012), but NMR was

Table 2. Multivariable Linear Regression Model of Log-Transformed Nicotine Metabolite Ratio as a Function of Opioid Use, Demographics, and Tobacco Use (*N* = 129)^a

	<i>b</i> Coefficient ^b	SE	<i>p</i> value
Opioid use (vs. nonuse)	0.29	0.11	.012
Cigarettes per day (CPD)	0.002	0.01	.832
Menthol smoking (vs. non-menthol)	0.13	0.11	.212
Race/ethnicity (vs. white)			
Black	−0.68	0.13	<.001
Hispanic/Latina	−0.18	0.10	.066
Other/refused	−0.89	0.26	.001

SE = standard error.

^aPredictors: opioid use, CPD; covariates: menthol smoking, race/ethnicity.

^bWe reported the unstandardized regression coefficient.

not associated with CPD. Black (*b* = −0.68, SE = 0.13, *p* < .001) and participants who answered “other” race or refused to answer (*b* = −0.89, SE = 0.26, *p* = .001) had a slower rate of metabolism than whites. Menthol smoking was not associated with NMR.

In the analysis of opioid type, methadone-maintained smokers had a faster nicotine metabolism rate than non-OU smokers (*b* = 0.32, SE = 0.14, *p* = .024), adjusting for CPD, menthol smoking, and race/ethnicity (data not shown). No other types of opioids were associated with NMR.

Discussion

In this preliminary study, we found that OU was associated with NMR among pregnant smokers. Specifically, although cotinine levels

were similar among smokers irrespective of OU, 3HC levels were higher among opioid-using smokers. Our results raise the possibility that OU, and methadone maintenance in particular, increases the rate of nicotine metabolism in pregnant smokers.

The novel association of higher NMR with OU, particularly methadone maintenance, during pregnancy is consistent with the reported clinical finding that nicotine dependence is greater in opioid-dependent smokers.⁴ In human liver cell lines, methadone strongly and dose-dependently induces the expression of *CYP2B6*, *CYP3A4*, *UGTA1*, and *MDR1* through activation of the PXR and constitutive androstane receptors.⁸ Although several drugs that induce *CYP2A6* also activate these nuclear receptors,⁶ to our knowledge, whether methadone or opioids induce *CYP2A6* through PXR- and/or constitutive androstane receptor-dependent mechanisms has not been examined. Further, metabolic and hormonal changes occur during both pregnancy and OU. Cholesterol levels are increased by 25%–30% during pregnancy,¹¹ and are elevated with chronic OU as part of a metabolic syndrome profile.¹⁰ Because PXR activity is increased by cholesterol metabolites and is necessary for cholesterol and hormone detoxification,¹² a downstream effect could be activation of *CYP2A6*.¹⁶ Also, animal studies have shown that opioid-treated animals have increased cholesterol accumulation and reduced synthesis of primary bile acids and recovery of secondary bile acids in the liver.¹³ This scenario would be expected to increase liver AKR1D1 expression, a gene postulated to be a regulator of nicotine metabolism.⁶ Finally, serum prolactin levels are markedly increased during pregnancy and with OU; whether this hormone could influence nicotine metabolism during pregnancy is unknown.

Although *CYP2A6* encodes the major enzyme in nicotine metabolism, *CYP2B6* (*4 haplotype) has also been associated with NMR, potentially because of being in linkage disequilibrium with functional *CYP2A6* variants.¹⁷ *CYP2B6* variation has also been associated with NMR in a genome-wide association study.¹⁸ A noncoding polymorphism in *CYP2B6* (rs8109525) previously associated with smoking cessation is also associated with nicotine metabolism.¹⁹ Although *CYP2B6* has a less prominent role (compared to *CYP2A6*) in metabolizing nicotine to cotinine, nicotine to cotinine metabolism could be amplified when *CYP2B6* is induced by opioids or by estrogen during pregnancy. However, we saw higher 3HC, but not cotinine, levels with OU, which could be explained by greater conversion of the formed cotinine to 3HC by *CYP2A6*, which is also increased in pregnancy. Future work could aim to disentangle the relative impacts of *CYP2A6* and *CYP2B6* on nicotine metabolism in pregnancy in the context of OU.

Although in our analysis an association with NMR was statistically significant only for methadone, the small number of buprenorphine users limited our ability to compare the effect of these maintenance therapies. A study of nicotine metabolism in nonpregnant smokers showed no effect of buprenorphine maintenance dose on NMR.¹⁵ Different opioids could influence nicotine metabolism differently. For example, an in vitro study found that buprenorphine modestly inhibited *CYP2B6*.²⁰ Contrary to previous studies,^{6,15} we found no association of NMR with CPD. However, not all studies have found associations between NMR and CPD in women,⁷ potentially underscoring the importance of non-nicotine factors for smoking in women.

Our study was limited by the small number of opioid-using smokers. It is also possible that the observed difference in nicotine metabolism resulted from confounding factors that were not measured in our study. Although we controlled for some of the most

well-known confounders, a prospective study is needed to validate and extend our findings. In addition, reliance on available medical records and participant interviews for duration of prescribed OU was a limitation of the study. Lastly, we relied on self-reported OU. Some women might not have reported their OU accurately, resulting in their inclusion in the nonuse group. This could have biased the results toward the null.

In summary, we found that pregnant smokers who used opioids, and in particular those maintained on methadone, had a higher rate of nicotine metabolism than pregnant smokers who did not use opioids. Our results are preliminary, and further studies are needed to replicate these findings, examine mechanisms, and evaluate their potential impact on smoking cessation.

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