Associations of Demographic Factors and Tobacco Use With Progesterone and Estradiol During Pregnancy

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Abstract

Objective: To evaluate the association of biochemically validated prenatal tobacco use with serum progesterone and estradiol in the second trimester of pregnancy, controlling for demographic and personal factors.

Study design: This secondary analysis of a multicenter longitudinal study included 114 women with singleton pregnancies. Multiple regression analysis assessed whether prenatal tobacco use was related to hormone levels during the second trimester, controlling for covariates (age, body mass index, and race or ethnicity, with gestational age added to subsequent models).

Result: In the initial regressions, tobacco users had significantly lower progesterone level compared with nonsmokers ($p = .037$), while estradiol was unrelated to prenatal tobacco use. Women with greater body mass index also had significantly lower progesterone ($p = .028$), but body mass index was unrelated to estradiol. With gestational age as an additional covariate, prenatal tobacco use was no longer a significant predictor of progesterone, but both body mass index and gestational age were significant ($F = 10.6$, $p < .001$, $R^2 = 0.35$). For estradiol, the overall regression of estradiol on age, body mass index, and race or ethnicity was not significant ($F = 1.2$, $p = .31$). With gestational age added to the model, the overall model was significant ($F = 7.2$, $p < .001$, $R^2 = 0.27$).

Conclusion: This study provides additional evidence that prenatal tobacco use may influence lower serum progesterone during the second trimester. This is of particular concern given the link between depressed progesterone activity and risk for preterm birth.

Keywords

progesterone, estradiol, prenatal tobacco use, nicotine, second trimester

Introduction

Smoking during pregnancy increases the risk of adverse outcomes, including impaired placental attachment and function, miscarriage, delayed fetal lung and brain development, stillbirth, preterm birth, low birth weight, and Sudden Infant Death Syndrome (Centers for Disease Control and Prevention, 2016; Dietz et al., 2010). Consistent with the increased likelihood of adverse events, smoking during pregnancy results in higher neonatal medical costs: In the United States, smoking-attributable health-care costs for neonates are

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approximately $366 million per year (Maurice, Kahende, Trosclair, Dube, & Husten, 2008). This is a major public health concern given that 12% of women in the United States report smoking during pregnancy (Tong et al., 2013), and prenatal smoking rates in many countries exceed 20% to 30% (World Health Organization, 2009).

Many biological and biochemical markers have been shown to be affected by smoking, and some of these have been linked to adverse outcomes during pregnancy. The level of the hormone progesterone is associated with smoking and is a biochemical indicator of potential for pregnancy complications. During pregnancy, progesterone is produced first by the corpus luteum and then the placenta; this hormone maintains the uterine lining to carry a fetus to term. When progesterone is low, the risk of miscarriage or preterm birth (PTB) increases as the placenta is identified by the maternal immune system as foreign tissue. Piasek, Blanusa, Kostial, and Laskey (2001) found that maternal smoking reduced placental progesterone content, which carries risks for both the infant and the mother.

Estradiol is another hormone that is crucial during pregnancy, though its connection to smoking is debated. At least one study has reported that serum estradiol levels are not affected by maternal smoking (Windham, Mitchell, Anderson, & Lasley, 2005), while another concluded that smoking can reduce the efficacy of the hormone (Mueck & Seeger, 2005). Although smoking has long been associated with an antiestrogenic effect, there is now an established pathway in the liver that may account for decreased bioavailability at estrogenic targets (Michnovicz, Hershcopf, Naganuma, Bradlow, & Fishman, 1986). However, estradiol has been shown to be crucial for maintaining a pregnancy, and its levels increase through trimesters (Mazor et al., 1994; Punnonen & Lukola, 1981). It also serves the important regulator of progesterone throughout the course of the pregnancy (Smith et al., 2009; Tal, Taylor, Burney, Mooney, & Giudice, 2015).

The purpose of this study was to evaluate the association of biochemically validated prenatal tobacco use with serum progesterone and estradiol in the second trimester of pregnancy, controlling for demographic and personal factors. We hypothesized that pregnant smokers would have lower progesterone than their nonsmoking peers, but we were unsure of association between prenatal tobacco use and estradiol.

Methods

Design and Sample

This was a secondary analysis of a prospective, multicenter study evaluating cytokine expression across trimesters in singleton pregnancies. The primary objective of the original study did not consider assessing the association of biochemically validated tobacco use with serum progesterone and estradiol during the second trimester of pregnancy, which is the purpose of this study. The institutional review boards of the University of Kentucky and the University of Virginia approved the original study and the modification for this study. This secondary analysis is based on the second trimester assessment of the longitudinal study, as this timepoint included measurement of the key analysis variables, namely, validated tobacco use, progesterone, and estradiol. Women aged 16 years and older with singleton pregnancies were recruited by research nurses or nurse practitioners into the study during the first trimester at a prenatal care visit to university-affiliated prenatal clinics in Kentucky and Virginia. Exclusion criteria were history of diabetes, heart disease, autoimmune disease, or HIV; indication of drug abuse during the second or third trimesters; diagnosis of sexually transmitted infection; or multifetal pregnancy or pregnancy incompatibility with life. Participants were informed of possible risks and their right to leave the study at any time without penalty, and 399 women agreed to participate. Upon the completion of each appointment at which data were collected, participants were given a $20 gift card. If participants completed all four appointments, they were given an additional $20 gift card, for a total of $100.00 in possible compensation for study participation.

Patients ($N = 114$) were included in this analysis if serum progesterone, estradiol, and urine cotinine measurements were obtained in the second trimester. Women on progestogen therapy (17-OHPC or vaginal progesterone) were excluded. Data were collected from January 2008 to December 2013. Demographics were collected at a first-trimester prenatal appointment at which time prenatal tobacco use was also confirmed via urine cotinine.

Measures

Personal characteristics. Demographic factors included age (in years) and race or ethnicity (with five options, including Hispanic). Given the limited numbers of women in each racial or ethnic minority, this variable was categorized as "White or non-Hispanic" or "Other." We also determined body mass index (BMI) using height and weight recorded in the baseline (first trimester) evaluation. Gestational age at the time of progesterone and estradiol assessment (second trimester) was determined relative to the ultrasound examination conducted in the first trimester.

Biochemical validation of tobacco use. The baseline (first trimester) urine samples were tested using NicAlert® strips. NicAlert® has been shown to be a valid biochemical indicator of smoking status in adults (Nymox, 2016).

A score of 3 or higher on this test is indicative of a urine cotinine value of 100 ng/mL or greater; this cutoff was used to determine tobacco use. NicAlert® cutoffs for smoking validation are consistent with previously reported urine cotinine ranges, including among pregnant women (Bernert, Harmon, Sosnoff, & McGuffey, 2005; Higgins et al., 2007). The first-trimester cotinine assessment was used for this measure, both to capture any prenatal exposure to active smoking and to acknowledge that most women who are still smoking at that point of their pregnancy continue to do so throughout the prenatal period; in our study, 86% of those who were smoking at the first-trimester assessment were still smoking during the second trimester (Ashford et al., 2018).

Hormones. Five cubic centimeters of blood were drawn into gold top tubes, centrifuged, and transported to the Reproductive Endocrinology Laboratory at the University of Kentucky. Serum was frozen until assayed. Estradiol and progesterone were assayed by a solid-phase, competitive chemiluminescent enzyme immunoassay using an Immulite 1000 (Siemens Healthcare Diagnostics, Los Angeles, CA) according to the manufacturer's recommendations. Serum and alkaline phosphatase-labeled hormone were added to antibody-coated beads which were then incubated at 37°C for up to 70 minutes. Test units were washed after incubation, alkaline phosphatase substrate was added, and the samples were incubated at 37°C for 10 minutes. Counts per second for each sample were converted to analyte concentrations using stored master curves. The assay sensitivities were as follows: estradiol (15 pg/mL) and progesterone (0.2 ng/mL). The intraassay and interassay coefficients of variation were routinely between 5% and 8% and 10% and 13%, respectively.

Biosample data are maintained by the Center for Clinical and Translational Science at the University of Kentucky. Inquiries about other data should be directed to the principal investigator and first author.

Data Analysis

Descriptive statistics, including means and standard deviations or frequency distributions, were used to summarize the study variables. Multiple regression analysis was used to test for an association between prenatal tobacco use and second-trimester hormone levels, controlling for age, BMI, and race or ethnicity; a second set of models, with one for each outcome, also included gestational age as an additional covariate (Singer, 1998). This second set of models was considered to determine if the significant association observed in the initial model between tobacco use and progesterone would remain significant when controlling for gestational age, a known correlate of progesterone (Tal et al., 2015). In addition,

the second model for estradiol was considered to evaluate whether any of the variables would predict this outcome; specifically, this model was to test whether the expected positive association between gestational age and estradiol would emerge, controlling for the other variables in the model. Variance inflation factors gauged the presence of multicollinearity in the regression models. Data analysis was done using SAS v. 9.4, with an alpha level of .05 used throughout.

Results

The average age of participants was 26.9 ($SD = 5.5$) and average BMI was 26.7 ($SD = 6.9$; see Table 1). Most of the participants were White or non-Hispanic (80%). Nearly one fifth were biochemically validated as smokers (19%). The average gestational age at the time of the second trimester assessment of hormones was 20.4 weeks ($SD = 3.1$). The average progesterone level was 57.1 ($SD = 19.3$), while mean estradiol was 7.8 ($SD = 4.9$).

Results of the multiple linear regression models are displayed in Table 2. With age, BMI, race or ethnicity, and confirmed tobacco use in the model, the overall regression of progesterone on these variables was significant ($F = 3.12$, $p = .018$). These personal factors and prenatal tobacco use accounted for 11% of the variability in second-trimester progesterone. Although progesterone level was not associated with age or race or ethnicity, for each one-unit increase in BMI, the average progesterone value decreased by more than one-half point (i.e., −.58 change for each one-unit increase in BMI). Compared with nonsmokers, active smokers had an average progesterone level that was more than 10 points lower (i.e., −10.32 difference in progesterone for

Table 1. Descriptive Summary of Demographic Variables, Smoking Status, and Hormones ($N = 114$).

	Mean (SD) or n (%)
Age	26.9 (5.5)
BMI	26.7 (6.9)
Race or ethnicity	
White or non-Hispanic	91 (79.8%)
Other	23 (20.2%)
Prenatal tobacco use	
Yes	22 (19.3%)
No	92 (80.7%)
Second trimester gestational age (weeks)	20.4 (3.1)
Progesterone	57.1 (19.3)
Estradiol	7.8 (4.9)

Note. BMI = body mass index.

Table 2. Tests of Hormone Differences by Prenatal Tobacco Use, Controlling for Demographic Factors ($n = 107$).

Variables in model	Progesterone			Estradiol		
	Est. b (SE b)	Std. β	p	Est. b (SE b)	Std. β	p
Age	0.176 (0.34)	0.05	.61	−0.114 (0.09)	−0.13	.20
BMI	−0.578 (0.26)	−0.21	.028	−0.039 (0.07)	−0.06	.56
White or non-Hispanic	−5.941 (4.59)	−0.12	.20	−1.872 (1.20)	−0.15	.12
Prenatal tobacco use	−10.326 (4.88)	−0.21	.037	−0.980 (1.28)	−0.08	.45
Model fit statistics:	$F = 3.1$, $p = .018$; $R^2 = .11$			$F = 1.2$, $p = .31$; $R^2 = .05$		
Age	0.200 (0.29)	0.06	.50	−0.086 (0.07)	−0.10	.25
BMI	−0.554 (0.22)	−0.21	.014	−0.030 (0.06)	−0.05	.60
White or non-Hispanic	−2.393 (3.98)	−0.05	.55	−1.586 (1.03)	−0.14	.13
Prenatal tobacco use	−5.407 (4.19)	−0.11	.20	−0.268 (1.17)	−0.02	.82
Gestational age	3.050 (0.50)	0.51	<.001	0.672 (0.13)	0.46	<.001
Model fit statistics:	$F = 10.6$, $p < .001$; $R^2 = .35$			$F = 7.5$, $p < .001$; $R^2 = .27$		

Note. BMI = body mass index; Est.: estimated.

the comparison of smokers to nonsmokers). When gestational age was added to the model, prenatal tobacco use became nonsignificant, but both BMI and gestational age were significant. The overall model was significant ($F = 10.6$, $p < .001$), and the R^2 for the model with gestational age added was 0.35. The decrease in progesterone with each one-unit increase in BMI was relatively consistent between the two models, and for the second model, the increase in progesterone for each additional week of gestational age was approximately 3 units.

With age, BMI, and race or ethnicity in the model, the overall regression of estradiol on these variables was not significant ($F = 1.2$, $p = .31$). Consistent with a nonsignificant regression, none of the potential predictors included in the model were significantly associated with estradiol level (Table 2). With gestational age added to the model, the overall model was significant ($F = 7.2$, $p < .001$). The only significant predictor was gestational age, but this and the other variables in the model accounted for 27% of the variability in estradiol. For each 1-week increase in gestational age, there was an average increase in estradiol of 0.67 units. The variance inflation factors for both sets of models were all less than 2, suggesting that the model parameters were not influenced by multicollinearity.

Discussion

Progesterone was found to be significantly lower in women who were active smokers compared with nonsmokers, when measured in the second trimester of pregnancy. This is consistent with the literature that has reported lower serum progesterone levels among women who smoke in early pregnancy (Soldin, Makambi, Soldin, & O'Mara, 2011; Toriola et al., 2011).

Soldin et al. (2011) further explored the relationship between tobacco exposure and progesterone and estradiol, also confirming lowers levels of both steroids in early pregnancy.

The found association between BMI and lower progesterone levels is also consistent with past research. Goh, He, Allen, Malhotra, and Tan (2016) found significant differences in serum progesterone in early pregnancy among underweight, normal weight, overweight, and obese women, when adjusting for age, multiparity, gestational age, timing of measurement, and, notably, smoking. Further, smoking has been found to be associated with higher BMI in White women as early as adolescence (ages 12–18 years; Young et al., 2015). It is likely that both smoking and higher BMI, as well as their combined effects, result in lower serum progesterone, which is potentially deleterious to maternal and fetal health during pregnancy.

Other studies have found a nonlinear relationship between BMI and estradiol (Ziomkiewicz, Ellison, Lipson, Thune, & Jasienska, 2008). Rehman, Hussain, and Faraz (2012) found that obese (BMI $\geqslant 26$) women had significantly lower estradiol than normal weight (BMI = 18–22.9) women, though overweight women (BMI = 23–25.9) had higher estradiol levels than normal weight participants. However, Jones et al. (2013) found a positive relationship between estradiol and BMI in a sample of postmenopausal women: Over 5 years, decreases in BMI were associated with decreases in estradiol.

Previous studies have shown higher maternal BMI to be associated with negative birth outcomes, including increased prevalence of indicated preterm delivery, as well as stillbirth, neonatal death, and birth defects (Koullali, Nijman, Mol, & Pajkrt, 2016; Vasudevan,

Renfrew, & McGuire, 2011). Clayborne, Giesbrecht, Bell, and Tomfohr-Madsen (2017) found that maternal weight mediated associations between neighborhood socioeconomic status and both large for gestational age births and macrosomia, which can necessitate preterm delivery (Spong et al., 2011). Lower serum progesterone, especially among women who smoke, is a possible mechanism for associations between higher BMI and preterm birth and related risks.

During pregnancy, estradiol varies with gestational age and tobacco use. A limited numbers of studies have explored associations between tobacco use and estradiol level in pregnant women and women of childbearing age. In nonpregnant women, serum estradiol measured in the follicular phase has been shown to be significantly higher in women who reported smoking a pack or more of cigarettes per day when compared with nonsmokers (Caserta et al., 2013; Zumoff et al., 1990). Estradiol levels in pregnant smokers have varied effects based on gestational age. Early in pregnancy, estradiol levels were significantly depressed in smokers (Soldin et al., 2011); on the other hand, Petridou et al. (1990) reported a marginally negative relationship between prenatal smoking and estradiol levels in the latter second trimester. Inconsistencies in measurement of smoking status, whether verified by cotinine or self-report, could also contribute to mixed results.

Conclusion

This was among the first studies to prospectively examine the relationship of confirmed tobacco use during pregnancy with serum progesterone and estradiol. This study provides additional evidence that prenatal tobacco users may have lower serum progesterone during the second trimester of pregnancy. This is of particular concern given the link between depressed serum progesterone levels and risk for preterm birth. Additional research is needed to establish best practices on biosample media (urine, serum) and timing of collection of estradiol to further evaluate the association between prenatal tobacco use and estradiol while examining whether any association may be linked to risk of adverse pregnancy outcome.

Declaration of Conflicting Interests

Funding

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