Original investigation

Maternal Smoking and Newborn Cytokine and Immunoglobulin Levels

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Abstract

Introduction: Prenatal smoking exposure may lead to permanent changes in neonatal inflammation and immune response that have lifelong implications, including increased risks for atopy and respiratory disorders.

Methods: The effect of maternal smoking on neonatal biomarkers of inflammation and immune response was assessed among 3459 singletons and twins in the Upstate KIDS Study. The following inflammatory biomarkers were measured using newborn dried blood spots (DBSs): interleukin (IL)-1 α , IL-1 receptor antagonist, IL-6, IL-8, C-reactive protein, and tumor necrosis factor alpha. Immunoglobulins (IgE, IgA, IgM, and IgG subclasses) were also assessed. We used generalized estimating equations to calculate mean differences (β) in biomarker levels by timing of pregnancy smoking, cigarette load, and secondhand smoke exposure after adjusting for sociodemographic and lifestyle factors including maternal body mass index.

Results: Of the 344 (12%) women reporting smoking during pregnancy, about 40% continued throughout pregnancy and 13% reported smoking more than 1 pack per day. After covariate adjustment and Bonferroni correction for multiple comparisons, maternal smoking throughout pregnancy remained significantly associated with increased levels of IL-8 ($\beta = 0.20$, 95% confidence interval: 0.07, 0.32; $p < .003$). No significant associations were found with cigarette load or secondhand smoke exposure. Higher IgG3 levels were also associated with maternal smoking throughout pregnancy, although the association became nominally significant after adjustment for covariates ($\beta = 0.09$; 95% confidence interval: 0.0007, 0.17; $p < .05$).

Conclusions: Maternal smoking throughout pregnancy was independently associated with increased IL-8 levels in newborns. Importantly, neonates of women who stopped smoking anytime in pregnancy did not have increased IL-8 levels.

Implications: This study evaluated a range of inflammatory biomarkers and immunoglobulins in association with maternal smoking and timing/duration of smoking along with secondhand smoke exposure. By using DBSs, we present data from a large cohort of children born in Upstate New York. Our findings suggest that early differences in immunoregulation of neonates exposed to maternal smoking for full duration in utero may already be detected at birth.

Introduction

In 2009, 25% of women aged 18–44 years reported smoking 3 months prior to pregnancy in the United States.¹ Of these women, 46% quit smoking before or during pregnancy.¹ Maternal smoking during pregnancy is associated with adverse perinatal outcomes and long-term health implications, including childhood cancers, asthma, and other respiratory disorders, for children exposed in utero.^{2–4} Additionally, secondhand smoke exposure has been found to have similar, albeit less pronounced adverse effects on fetal and child health compared to active smoking habits.⁵ Maternal smoking is an important modifiable risk factor that remains a public health concern.

Despite evidence of the effect of maternal smoking on child health and development, little is known about the effects of cigarette smoking during pregnancy on neonatal inflammatory and immune responses. Studies suggest that both the intrauterine environment and genetic factors modify the relationship between maternal and infant cytokine responses, making it difficult to interpret the independent effect of maternal smoking.⁶ Cigarette smoking is known to exhibit both immunosuppressive and proinflammatory properties within smokers,⁷ and this is further complicated by immunologic and inflammatory changes that occur in pregnant women.⁸ Several retrospective studies assessed the immunological effects of maternal smoking on neonates by measuring cytokines and other inflammatory biomarkers but among small clinical populations using cord blood or other retrieval techniques.^{9–13} For example, using cord blood mononuclear cells, Noakes et al. observed both suppressive and proinflammatory cytokine responses to allergens in neonates of pregnancy smokers compared to neonates whose mothers never smoked.⁹ Similarly, in studies that measured infant cord blood immunoglobulin levels, the effect of maternal smoking is not well understood due to conflicting findings, with some studies reporting increased neonatal responses^{11,14–16} and others reporting no effect on neonatal immunoglobulins.^{17,18}

There is also a poor understanding of whether biomarkers of neonatal inflammation or immune response change with maternal smoking duration either before or during pregnancy. To our knowledge, no studies have looked at the timing of smoking cessation during pregnancy and cytokine levels in neonates.

To understand whether the timing and duration of prenatal tobacco exposure alter inflammation and immune responses in early life, we evaluated the effects of maternal smoking with respect to timing, cigarette load, and secondhand smoke exposure on neonatal inflammatory biomarkers and immunoglobulins using newborn dried blood spots (DBSs) from a population-based cohort.

Materials and Methods

Study Population

We used data from the Upstate New York Infant Development Screening Program (Upstate KIDS) study, a population-based cohort of children from New York State (excluding New York City) born between September 2008 and December 2010. Originally designed to study the effects of infertility treatment on child development, the study oversampled on children conceived by infertility treatment as well as on multiple gestations.¹⁹ Triplets and quadruplets ($n = 134$) were excluded from the current analysis due to small sample size. Children of women who did not return a 4-month postpartum questionnaire ($n = 192$) were excluded due to missing smoking information. Children of women who did not provide consent for the

use of residual DBS ($n = 2286$) were excluded. Lastly, $n = 100$ children were excluded due to missing/inconsistent maternal smoking information, making the final study population $n = 3459$ children (born to 2903 mothers). The study was approved by the Institutional Review Boards (IRBs) of the University at Albany (08-179) and the New York State Department of Health (07-097), which served as the designated IRBs for the Upstate KIDS Study under a reliance agreement with the National Institutes of Health. Written and informed consent was provided by all participants. Additional parental consent was obtained at 8 months postpartum for the use of residual DBS. Characteristics between parents who consented for DBS use and those who did not were comparable.²⁰

Maternal Smoking

Maternal smoking was reported on the questionnaire administered at 4 months postpartum. Any incomplete maternal smoking information was obtained from birth certificates ($n = 56$ women, 2%). Women were asked about any smoking prior to pregnancy (“Have you smoked more than 100 cigarettes/5 packs in your lifetime?”), active and passive smoking during pregnancy (“During this pregnancy, about how many cigarettes did you smoke per day, on average?”), “Did you stop smoking at any point during this pregnancy?”, “During this pregnancy, did you live with a smoker?”), and smoking habits at 4 months postpartum (“Do you smoke at all now? About how many cigarettes per day do you currently smoke on average?”). Participants were provided with categorical choices for both responses to stopping time (i.e., by trimester) and number of cigarettes smoked (i.e., less than a pack, about a pack, 1–2 packs, or 3 or more packs per day). Nonsmokers and smokers who quit prior to pregnancy served as the reference group for all analyses. Several women who quit before pregnancy recommenced by 4 months postpartum ($n = 74$ women, 2.5%). We excluded this group in order to ensure a clean reference group of women who, with a degree of certainty, were not smoking during pregnancy.

Information on timing and dosage of maternal smoking was available for $n = 2897$ and $n = 2903$ women and their $n = 3452$ and $n = 3459$ children, respectively. Women who reported smoking during pregnancy were further categorized into: (i) stopped within the first trimester, (ii) stopped after the first trimester, or (iii) never stopped. The decision to categorize exposure timing based on the first trimester was due to the low rate of smoking cessation (2%) during the second and third trimesters. Cigarette load was dichotomized at < 1 pack (20 cigarettes)/day or ≥ 1 pack (20 cigarettes)/day due to the small numbers of participants responding to the heavier smoking categories (i.e., two women reported smoking 1–2 packs/day and one woman reported smoking 3 or more packs/day during pregnancy).

Additionally, we looked at secondhand smoke exposure in combination with maternal smoking. Infants born to women who did not smoke and did not live with a smoker during pregnancy served as the reference group. Exposure groups were created for infants born to women who did not smoke during pregnancy but lived with a smoker at the time, women who smoked during pregnancy but did not live with another smoker, and women who smoked during pregnancy and lived with another smoker. The final sample size for our analysis of secondhand smoke exposure was $n = 2857$ mothers and their $n = 3402$ children ($n = 57$ infants missing information on secondhand smoking).

Inflammatory Biomarkers and Immunoglobulins

As part of the state’s Newborn Screening Program, DBSs are obtained 2–3 days after birth from a heel-prick and five drops of whole blood

are placed on filter paper and then stored at low temperatures until further analysis. Particularly useful in newborn screening, DBS samples provide a noninvasive method for detecting levels of biomarkers representative of endogenous and environmental exposures. For population-based cohorts, DBS can serve as a convenient method to retrieve biological specimen and reduces the cost of immunoassay analysis of certain inflammatory markers in whole blood.²¹ Newborn DBSs were stored at 4°C and punches of the residual spots were extracted using methods previously described for other analytes measured from DBSs in our study.^{20,22,23}

The biomarkers of interest were selected a priori based on existing knowledge of biological processes involved in systemic inflammation and immune function.²⁴ These included four interleukins (interleukin 1 alpha [IL-1α], interleukin 1 receptor antagonist [IL-1ra], interleukin-8 [IL-8], and IL-6), C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), and immunoglobulins (Igs) for immune function (i.e., IgE, IgA, IgM, and IgG subclasses). All biomarkers except for IgE were measured as part of Kit A, Obesity, and Ig multiplex panels (R&D Systems, Minneapolis, MN; For Ig panel: HGAMMAG-301K-06, EMD Millipore Corp., Billerica, MA) using Luminex¹⁰⁰ analyzer with xPONENT 3.1 software (Luminex System, Austin, TX). IgE concentrations were measured using ELISA (Cat 3810-1H-20, MABTECH Inc., Cincinnati, OH). Intra-assay coefficients of variation (CVs) were 14.0% for IL-1α, 10.1% for IL-1ra, 16.9% for IL-6, 11.5% for IL-8, 8.6% for CRP, and 8.4% for TNF-α. Intra-assay CVs for all Igs ranged from 4.8% to 12.0%. TNF-α and IL-6 concentrations had high percentages of zero values (77% and 49%, respectively). Zero values indicated that the measured concentration for TNF-α or IL-6 was lower than the background machine reading, and no trace of the biomarker was present at the time of measurement. All analyses used machine-read values without censoring to overcome latent biases.²⁵ Measurement error introduced from the processing of multiple batches was removed using ComBat in the R software package.^{26–28}

We assessed these biomarkers individually and in combination as an inflammatory “score.” Igs were analyzed individually. Each infant was assigned one point for a value above the population median level for each proinflammatory biomarker (IL-1α, IL-6, IL-8, CRP, and TNF-α) and for the anti-inflammatory biomarker, IL-1ra, one point was given for a value below the median level. The points for each inflammatory biomarker were then summed in order to derive an overall inflammation score with scores ranging from 0 to 6 for each child.²⁹ A modified inflammation score was also calculated excluding TNF-α and IL-6 due to the large number of zero values for these two biomarkers.

Covariates

Information on sociodemographic characteristics such as maternal race and ethnicity, education, marital status, health insurance, age, prepregnancy body mass index (BMI), alcohol use during pregnancy (yes/no), any dietary supplementation during pregnancy (yes/no), any infertility treatment for the index pregnancy (yes/no), and parity was obtained from the 4-month postpartum questionnaire or birth certificates. Infant characteristics including gender, plurality, birth weight, and gestational age were obtained from birth certificates.

Statistical Analyses

Chi-square and independent sample *t* tests were used to determine differences in baseline characteristics by maternal smoking status in pregnancy.

Analyses were performed in all singletons and both twins (*n* = 3459). Generalized estimating equations (GEEs) with robust standard errors were used to estimate associations between maternal smoking and continuous levels of individual inflammatory biomarkers and Igs, as well as the overall inflammation score. GEE was specifically used to account for correlation among twins. Due to the large number of zero values for TNF-α and IL-6, the two biomarkers were dichotomized at the zero concentration. GEE logistic regression models were used to evaluate these associations with maternal smoking. Biomarker values were transformed for normality before using complete Markov chain Monte Carlo data imputation for missing values. Biomarker values were back transformed after imputations. In the imputed data set, box-cox transformation was used to assess the optimal method of transformation for each biomarker conditional on their associations with maternal smoking. All biomarkers were log transformed in analyses. Missing values for prepregnancy BMI, alcohol use during pregnancy, private insurance, marital status, and parity were imputed using multiple imputations through creating 10 data sets based on a Bernoulli distribution with probabilities dependent on their observed distributions by type of infertility treatment.

We adjusted for maternal age, race and ethnicity, education, insurance, marital status, prepregnancy BMI, alcohol use during pregnancy, any dietary supplement use while pregnant, any infertility treatment, parity, and infant plurality.³⁰ Our aim was to estimate the overall effect of maternal smoking on cytokine levels in neonates. Hence, we did not adjust for mediators such as pregnancy complications or maternal/neonatal infections because that may cause over-adjustment bias in analyses for the association between smoking and inflammation.³¹ Similar to previous studies using data from this cohort, sampling weights using New York State vital record information of all births from 2008 to 2010 were applied to all models to account for the oversampling of twins and children conceived by infertility treatment.¹⁹ Additionally, we weighted models to account for DBS consent. In sensitivity analyses, GEE was used to test associations between the modified inflammation score and maternal smoking.

Estimates and 95% confidence intervals were reported for all biomarkers. To account for multiple hypothesis testing, a Bonferroni-corrected *p* value was set at *p* < .0036 (0.05/14) (i.e., 13 biomarkers and the overall score). Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

Results

About 12% of women smoked during pregnancy (Table 1), of whom 156 (43%) smoked throughout the entire pregnancy. Women who smoked during pregnancy were younger and more likely to be single and have a higher prepregnancy BMI than those who did not smoke. Smokers were also less likely to have private insurance, complete higher education, have taken dietary supplements during pregnancy, and have used infertility treatment. Infants born to women who smoked during pregnancy had lower birth weights compared with infants born to women who did not smoke in pregnancy. Distributions of inflammatory biomarker and immunoglobulin levels for mother-child pairs stratified by maternal smoking status are shown in Supplementary Table 1.

Unadjusted and adjusted mean differences as log unit changes in continuous measures of neonatal biomarkers and inflammation score by timing of smoking exposure in pregnancy are shown in Table 2.

Table 1. Baseline Characteristics by Maternal Smoking Status of Participants Consenting to Use of Dried Blood Spots in the Upstate KIDS Study

Characteristics ^a	N = 2903		p Value
	Smoking during pregnancy	No smoking during pregnancy	
	n (%)	n (%)	
Mothers	344	2559	
Age (y) ^b	27.1 (6.3)	31.7 (5.6)	<.001
Non-Hispanic White	295 (85.8)	2136 (83.5)	.28
Prepregnancy BMI (kg/m ²) ^b	27.8 (7.3)	26.8 (6.8)	.01
Privately insured	171 (49.7)	2147 (84.0)	<.001
Married	260 (77.2)	2345 (92.8)	<.001
College degree or higher	44 (12.8)	1613 (63.0)	<.001
Alcohol use during pregnancy	47 (13.7)	358 (14.0)	.87
Any dietary supplementation during pregnancy	227 (66.0)	2111 (82.5)	<.001
Any infertility treatment	32 (9.3)	911 (35.6)	<.001
Nulliparous	145 (42.3)	1180 (46.5)	.14
Infants ^c			
Males	183 (53.2)	1318 (51.5)	.56
Singletons	287 (83.4)	2030 (79.3)	.08
Birth weight (g) ^b	3120.1 (687.1)	3210.0 (674.6)	.02
Gestational age (wk) ^b	38.3 (2.3)	38.1 (2.4)	.31

BMI = body mass index; N = total number.

^aNumber of participants missing information for characteristics before analyses: 4 for prepregnancy BMI, 2 for insurance, 31 for marital status, 1 for alcohol use during pregnancy, 22 for parity.

^bData are presented as *n* (%) with the following exceptions: age (y), birth weight (g), gestational age (wk), and prepregnancy BMI (kg/m²) are reported as mean (*SD*).

^cTotal sample size is restricted to all singletons and one twin selected at random for a 1:1 ratio of mothers: infants.

After adjustment, only IL-8 levels were elevated among infants born to women who smoked for their entire pregnancy ($p = .003$). No other differences were statistically significant or noted after adjustment (i.e., IgG1, IgG3, and IgM).

Differences in neonatal biomarkers were not detected when we quantified the intensity of cigarette exposure during pregnancy (Table 3). IL-8 levels again exhibited a linear trend with increased cigarette load (p for trend = .009). Secondhand smoke exposure in addition to maternal smoking status did not produce significant differences in the measured biomarkers (Supplementary Table 2). For biomarkers dichotomized at the zero concentration (TNF- α and IL-6) for which odds ratios and 95% confidence intervals were calculated, no increased odds were found with maternal smoking (Supplementary Table 3). In sensitivity analyses, results were similar and nonsignificant between maternal smoking and the modified inflammation score (data not shown).

Discussion

To our knowledge, this is the largest study to use biomarkers measured from newborn DBSs to evaluate the effects of maternal smoking on neonatal inflammation and immune response. We found that timing of maternal smoking during pregnancy, specifically exposure to maternal smoking through the entire pregnancy, was associated with elevated IL-8 concentrations in neonates. Associations were also suggested between higher cigarette load and secondhand smoke exposure and elevated IL-8 levels in infants, but estimates were imprecise. No significant associations were found between maternal smoking and other DBS biomarker levels. Our results indicate that prenatal smoke exposure likely interferes with normal circulating IL-8 levels in neonates that appear to direct immune system changes shortly after birth.

Cytokines and Other Inflammatory Biomarkers

Cytokines act as biological mediators in cell-mediated (T-helper 1 [Th1]) and humoral (T-helper 2 [Th2]) immune system responses.³² Both pregnancy and cigarette smoke activate Th2 over Th1 responses.^{8,33} Predominant humoral immune system activation then triggers the release and inhibition of both proinflammatory and anti-inflammatory cytokines, with proinflammatory and immunosuppressive effects that manifest in the form of allergic and autoimmune reactions.⁷ Furthermore, smoking can impact the innate immune response that is involved in the regulation and production of key cytokine mediators, such as TNF- α and IL-8.³⁴ Some studies have shown that cigarette smoke in an individual increases levels of proinflammatory cytokines such as IL-1, IL-6, IL-8, and TNF- α , while decreasing levels of certain anti-inflammatory cytokines (i.e., IL-10).^{35,36} However, other studies report conflicting associations of lower IL-6 and TNF- α concentrations in smokers.^{33,37} Recent studies have shown that this suppression of certain proinflammatory cytokines is attributable to nicotine, a major component of cigarette smoke.^{7,38}

Despite the growing body of evidence on the immunological effects of tobacco smoke in adults, less is known about the effects of prenatal smoke exposure on circulating cytokine levels in neonates. Noakes et al. observed that maternal smoking was associated with higher Th2 activity as measured via increased IL-5, IL-9, and IL-13 levels in cord blood mononuclear cells.⁹ Another study by Noakes and colleagues concluded that the effect of maternal smoking on neonatal cytokine responses can also be mediated by toll-like receptor (TLR) function; more specifically, TLR2 pathways, which include IL-6, TNF- α , and IL-10, are attenuated in infants of smokers.¹⁰ Similarly, Latzin et al. reported decreased odds of cord IL-6 concentrations in infants born to smokers with a trend for lower IL-10 levels in cord blood.³⁹

Table 2. Mean Differences in DBS-Measured Neonatal Biomarkers by Timing of Smoking Exposure in Pregnancy

Neonatal biomarkers ^{a,b,c}	Stopped within the 1st trimester		Stopped after 1st trimester		Smoked for the entire pregnancy	
	N = 160		N = 72		N = 156	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Inflammation score	0.12 (-0.05, 0.29)	0.01 (-0.17, 0.18)	0.11 (-0.14, 0.35)	-0.002 (-0.25, 0.25)	0.09 (-0.09, 0.27)	0.01 (-0.18, 0.20)
CRP	0.11 (-0.01, 0.22)	0.03 (-0.08, 0.15)	0.08 (-0.06, 0.23)	0.01 (-0.13, 0.16)	0.12 (0.01, 0.24)	0.10 (-0.02, 0.21)
IL-8	0.06 (-0.04, 0.16)	0.04 (-0.06, 0.15)	-0.02 (-0.17, 0.13)	-0.03 (-0.18, 0.12)	0.20 (0.08, 0.32)	0.20 (0.07, 0.32)
IL-1ra	-0.06 (-0.15, 0.03)	-0.08 (-0.17, 0.02)	0.01 (-0.12, 0.13)	-0.04 (-0.16, 0.08)	0.08 (-0.02, 0.17)	0.05 (-0.05, 0.14)
IL-1 α	0.001 (-0.13, 0.13)	-0.01 (-0.15, 0.12)	0.04 (-0.17, 0.25)	-0.02 (-0.24, 0.20)	-0.003 (-0.14, 0.13)	-0.03 (-0.17, 0.12)
IgE	0.002 (-0.11, 0.12)	-0.01 (-0.13, 0.11)	-0.03 (-0.19, 0.14)	-0.03 (-0.19, 0.13)	0.00002 (-0.12, 0.12)	-0.01 (-0.13, 0.11)
IgA	0.07 (-0.01, 0.15)	0.06 (-0.02, 0.15)	0.03 (-0.09, 0.15)	0.03 (-0.09, 0.15)	0.03 (-0.05, 0.11)	0.05 (-0.03, 0.13)
IgM	0.02 (-0.06, 0.10)	0.02 (-0.06, 0.10)	0.12 (0.01, 0.24)	0.13 (0.01, 0.24)	-0.01 (-0.09, 0.06)	0.001 (-0.08, 0.08)
IgG1	-0.02 (-0.06, 0.02)	-0.02 (-0.07, 0.02)	0.07 (0.01, 0.14)	0.07 (0.001, 0.14)	0.02 (-0.03, 0.06)	0.02 (-0.03, 0.06)
IgG2	0.005 (-0.10, 0.11)	0.03 (-0.07, 0.14)	-0.04 (-0.18, 0.11)	0.01 (-0.14, 0.16)	-0.04 (-0.14, 0.06)	0.02 (-0.09, 0.12)
IgG3	-0.02 (-0.11, 0.07)	-0.07 (-0.16, 0.02)	0.17 (0.04, 0.30)	0.09 (-0.05, 0.22)	0.16 (0.08, 0.24)	0.09 (0.0007, 0.17)
IgG4	0.05 (-0.14, 0.23)	0.05 (-0.13, 0.24)	-0.01 (-0.27, 0.25)	-0.01 (-0.28, 0.26)	0.03 (-0.15, 0.22)	0.05 (-0.14, 0.25)

IL = interleukin; CI = confidence interval; CRP = C-reactive protein; DBS = dried blood spot; Ig = immunoglobulin; IL-1ra = IL-1 receptor antagonist; N = total number of infants.

^aTotal sample size includes all singletons and twins with information on timing of smoking exposure ($n = 3452$ infants born to 2897 mothers) in the Upstate KIDS birth cohort.^bInfants born to women who did not smoke during pregnancy served as the reference group ($n = 3064$ infants born to 2559 mothers).^cUnits: log ng/mL for all neonatal biomarkers except inflammation score (no units) and CRP (units: log mg/L).

Adjusted for maternal age, white race, attainment of college degree or higher, private insurance, marital status, alcohol use during pregnancy, any dietary supplementation during pregnancy, parity, infant plurality, prepregnancy body mass index, and any infertility treatment.

Statistically significant differences based on Bonferroni correction ($0.05/14 = p < .0036$) are in bold.

Table 3. Mean Differences in DBS-Measured Neonatal Biomarkers by Quantity of Cigarettes/Packs Smoked Per Day in Pregnancy

Neonatal biomarkers ^{a,b,c}	Smoked < 1 pack (20 cigarettes) per day		Smoked ≥ 1 pack (20 cigarettes or more) per day	
	N = 345		N = 50	
	Unadjusted	Adjusted	Unadjusted	Adjusted
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Inflammation score	0.10 (−0.01, 0.22)	0.004 (−0.12, 0.13)	0.19 (−0.11, 0.49)	0.11 (−0.19, 0.41)
CRP	0.10 (0.02, 0.18)	0.05 (−0.03, 0.13)	0.20 (0.001, 0.39)	0.17 (−0.02, 0.37)
IL-8	0.08 (0.01, 0.16)	0.07 (−0.01, 0.15)	0.20 (0.03, 0.38)	0.21 (0.03, 0.39)
IL-1ra	0.01 (−0.06, 0.07)	−0.02 (−0.09, 0.05)	0.05 (−0.10, 0.21)	0.02 (−0.14, 0.17)
IL-1α	0.002 (−0.09, 0.10)	−0.02 (−0.13, 0.08)	0.06 (−0.17, 0.29)	0.01 (−0.22, 0.25)
IgA	0.05 (−0.01, 0.10)	0.05 (−0.01, 0.11)	0.08 (−0.05, 0.22)	0.10 (−0.04, 0.24)
IgM	0.05 (−0.01, 0.10)	0.05 (−0.01, 0.11)	−0.07 (−0.21, 0.06)	−0.06 (−0.19, 0.08)
IgG1	0.01 (−0.02, 0.04)	0.01 (−0.02, 0.04)	0.03 (−0.05, 0.11)	0.03 (−0.05, 0.11)
IgG2	−0.02 (−0.09, 0.06)	0.03 (−0.05, 0.10)	−0.06 (−0.24, 0.11)	−0.02 (−0.19, 0.16)
IgG3	0.10 (0.03, 0.16)	0.03 (−0.04, 0.10)	0.05 (−0.09, 0.20)	−0.03 (−0.18, 0.11)
IgG4	0.08 (−0.05, 0.20)	0.09 (−0.04, 0.22)	−0.29 (−0.61, 0.03)	−0.28 (−0.60, 0.04)

IL = interleukin; CI = confidence interval; CRP = C-reactive protein; DBS = dried blood spot; Ig = immunoglobulin; IL-1ra, IL-1 receptor antagonist; N = total number of infants.

^aTotal sample size includes all singletons and twins with maternal smoking information ($n = 3459$ infants born to 2903 mothers) in the Upstate KIDS birth cohort.

^bInfants born to women who did not smoke during pregnancy served as the reference group ($n = 3064$ infants born to 2559 mothers).

^cUnits: log ng/mL for all neonatal biomarkers except inflammation score (no units) and CRP (units: log mg/L).

Adjusted for maternal age, white race, attainment of college degree or higher, private insurance, marital status, alcohol use during pregnancy, any dietary supplementation during pregnancy, parity, infant plurality, prepregnancy body mass index, and any infertility treatment.

In contrast with these studies using cord blood,^{9,10,39} we did not observe differences in DBS biomarker concentrations, with the exception of IL-8, by prenatal smoke exposure. Direct comparison of this study with previous reports should be done with caution as cytokine measurements in cord blood are highly influenced by perinatal factors, while in the present study we measured cytokines in DBS taken a few days after birth. The inconsistencies between our findings and associations of lower neonatal IL-6 and TNF- α with maternal smoking may also be attributable to differences in study samples, sample size, and minimal control of confounding in aforementioned studies. For example, previous studies lacked information on relevant confounders such as prepregnancy BMI or sociodemographic factors such as education and marital status. One study also adjusted for potential mediators, including gestational age and mode of delivery, and is therefore not reporting the total effect of smoking on neonatal immune function.¹⁰ Another possible explanation for why we did not observe associations with TNF- α and IL-6 may be due to degradation that has been reported to occur after 4–6 hours at room temperature.⁴⁰ However, studies measuring DBS biomarker concentrations have also reported low or undetectable levels for various cytokines and chemokines in infants from the general population,⁴¹ and therefore, might also explain why we did not observe population-based associations between maternal smoking and most DBS biomarkers compared to studies measuring cord blood levels. Importantly, we used DBSs collected from the statewide newborn screening program, which is a feasible, cost-effective tool for immunoassay analysis of inflammatory biomarkers in whole blood at proper storage techniques for population-based studies.^{21,42}

Interleukin-8

As a proinflammatory cytokine and chemotactic agent, IL-8 directs neutrophils and other immune cells to the site of inflammation or

cellular injury; IL-8 also contributes to mitogenic and angiogenic processes.^{43,44} Therefore, IL-8 production in pregnant women increases in the presence of infections (i.e., chorioamnionitis and neonatal sepsis) or other adverse pregnancy outcomes, which elicit similar responses such as preterm birth.^{45,46} Specifically in smokers, IL-8 production is enhanced and acts as a potential mechanism in the development of chronic obstructive pulmonary disease and lung cancers.^{47,48} In animal models, cigarette smoking has been shown to increase bronchial epithelial proinflammatory responses with respect to IL-8 secretion, which then induce pathological cellular injury.⁴⁹

In the present study, in-utero smoke exposure was found to impact neonatal inflammation with respect to increased DBS IL-8 levels. IL-8 does not appear to cross the placenta in detectable concentrations, and therefore, our finding suggests increased IL-8 levels are directly produced by the neonate as a result of in-utero smoke exposure.⁵⁰ Although less is known about the mechanistic actions by which maternal smoking influences neonatal IL-8 levels, it is possible that changes in fetal programming and potential insults occurring in utero, such as toxicant exposure, fetal hypoxia, and placental aberrations in protein metabolism, as a result of maternal smoking may alter long-term cytokine signaling in infants.

Accumulating evidence suggests that IL-8 may play a major role in enhancing the disease state of certain chronic pulmonary disorders such as asthma.⁵¹ One population-based study examining the inflammatory profile of neonates with impaired lung function at 4 weeks of life reported increased IL-8 levels in these infants.⁵² Another study found higher levels of IL-8 in newborns with increased risks of developing chronic lung disease regardless of preterm/term status.⁵³ Although previous research has explored IL-8 as a determinant of impaired lung function, evidence from our study supports the hypothesis that prenatal smoke exposure may impair cytokine signaling responsible for normal lung function later in life.

Immunoglobulins

Previous animal studies have shown that cigarette smoke exposure lowers serum Ig levels for IgA, IgG, and IgM in particular, but increases IgE levels—which is also suspected to occur in adult smokers.^{54,55} However, in neonates, previous smoking studies have either reported no change or increased concentrations of cord blood Igs in exposed versus nonexposed infants.^{11,14,16–18} We observed elevated IgG1, IgG3, and IgM levels in smoking groups with respect to timing of exposure and increased IgA levels with respect to secondhand smoke exposure, but these differences were not statistically significant after Bonferroni correction, and in the case of IgG3, after adjustment for confounders. Igs directly produced by the neonate include IgA, IgM, and IgE.⁵⁶ However, IgG is easily transferable from the mother to the fetus across the placenta.⁵⁷ Neonatal IgG production does not begin until 6 months after birth, and from the time of passive transfer by the mother in the few weeks leading to delivery until 6 months, IgG levels continue to fall as transferred maternal IgG is catabolized.⁵⁶ Therefore, DBS IgG levels might reflect maternal immune responses. In our study, similar to others, we cannot confirm DBS IgG levels are of neonatal origin, and therefore, IgG or other transferable biomarkers should be explored more closely. Normal placental transport of IgG subclasses may have also been affected by maternal smoking due to inflammation and a possible increase in the proportion of neutrophils associated with elevated circulating IL-8 levels. In line with Ownby et al. and Oryszczyn et al.^{17,18} but not Magnusson et al.¹⁶, we did not find any association between maternal smoking and neonatal IgE levels.

Strengths and Limitations

This study had several strengths. First, we used a population-based sample with singletons and twins, which improved the generalizability of our results. Second, we were able to control for many sociodemographic and lifestyle characteristics unavailable in smaller, clinical populations. Third, we used an overall inflammation score based on median biomarker levels, which did not assume normal distribution and was unaffected by outliers compared to other existing methods.

Nevertheless, our study is not without limitations. We used self-reported maternal smoking without validation from cotinine measurements. However, the prevalence of smoking in our cohort (12%) was comparable to that of other population-based studies using cotinine measurements, citing a smoking prevalence between 13% and 17% during pregnancy.^{17,58} In addition, a previous prospective study in the United States reported that cord blood cotinine concentrations were significantly correlated with self-reported smoking behaviors.¹⁷ We are also limited by questions pertaining to only cigarette smoking and did not ask mothers about other forms of tobacco/nicotine exposure such as chewing tobacco and electronic cigarettes. Selection bias might have arisen if the participants consenting to DBS use differed from participants without consent. However, since smoking status during pregnancy was not different between the two groups²⁰ this is less likely the case. Furthermore, we used sampling weights for DBS consent in our analysis. Although we tested multiple associations, we interpreted our results conservatively after applying Bonferroni correction. We cannot rule out the degradation of inflammatory biomarkers, especially IL-6 and TNF- α , as a limitation of the study. Nonetheless, this degradation which depends on time exposure to ambient temperature should occur at random, and therefore, should not introduce bias in the study. Lastly, the study is not representative of the U.S. population with participation of mostly non-Hispanic white families from a single state.

Conclusion

Findings from this population-based study reveal that prenatal smoke exposure can alter cytokine signaling in newborns with respect to IL-8, a proinflammatory chemokine linked to impaired lung function, respiratory disorders, and various pathological conditions. We believe our study is the first to provide population-based evidence on this association as measured with newborn DBS; therefore, it is important for future prospective studies to confirm our findings and follow newborns over time in order to assess the predictive utility of this biomarker for childhood cancers and respiratory disorders, especially ones persisting into adulthood that are linked to the overexpression of IL-8.

Supplementary Material

Supplementary data are available at *Nicotine & Tobacco Research* online.

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Declaration of Interests

None declared.

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