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Publication Date
2018-05-01

DOI
10.1016/j.ntt.2018.03.003

HHS Public Access

Author manuscript

Neurotoxicol Teratol. Author manuscript; available in PMC 2019 May 01.

Published in final edited form as:

Neurotoxicol Teratol. 2018 ; 67: 31–36. doi:10.1016/j.ntt.2018.03.003.

Prenatal and Postnatal Cigarette and Cannabis Exposure: Effects on Secretory Immunoglobulin A in Early Childhood

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Abstract

Aims—Secretory Immunoglobulin A (SIgA) plays a critical role in immune functioning by preventing pathogens from adhering to epithelial mucosa. Most infectious agents enter the body via mucosal surfaces, thus SIgA serves in the defense against respiratory, intestinal, and urinogenitary infections, as well as periodontal disease and caries. This study examined the possibility that pre- and postnatal exposure to cigarette and cannabis is associated with individual differences in Secretory Immunoglobulin A (SIgA) levels in early childhood.

Methods—Participants were 50 mother/infant (29 boys; 35% Caucasian) dyads recruited at their first prenatal appointment in a large northeastern community hospital in the United States. Repeated assessments of pre- and postnatal cigarette and cannabis were conducted beginning in the first trimester of pregnancy, using multiple methods (i.e., saliva, meconium, self-report). Infants were grouped into those prenatally exposed to either cigarette only (n=19), cigarette and cannabis (n=19), or with no prenatal substance exposure (n=12). At age 5 years, the children's saliva was collected and assayed for SIgA.

Results—There were group differences in SIgA levels as a function of prenatal exposure to cigarette and cannabis -- children in the cigarette only and the cigarette and cannabis groups had higher SIgA levels compared to the non-exposed children. Children who experienced the combination of postnatal exposure to cigarette and cannabis had higher levels of SIgA, even after accounting for prenatal exposures and other covariates relevant to immune system functioning.

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Conflict of Interest Statement

Disclosure statement:

In the interest of full disclosure, DAG is founder and Chief Scientific and Strategy Advisor at Salimetrics LLC (Carlsbad, CA) and Salivabio LLC (Carlsbad, CA). These relationships are managed by the policies of the committees on conflict of interest at the Johns Hopkins University School of Medicine and University of California at Irvine. Danielle Molnar, Rina Eiden, and Shannon Shisler have no conflicts of interest.

Conclusions—Prenatal and postnatal exposure to cigarette and cannabis may be associated with hyperactivity of mucosal immunity in early childhood. Links between cigarette and cannabis exposure and health problems in early childhood may be partially explained by prenatal and postnatal exposure-related changes in mucosal immunity.

INTRODUCTION

Maternal cigarette and cannabis use during pregnancy constitute a significant public health problem across the world as they are two of the most commonly used substances during pregnancy (El Marroun et al., 2008; Passey et al., 2014; USDHHS, 2014). The rates of use are as high as 27% for tobacco and 10% for cannabis in the United States, with the majority of these cannabis users being daily users (Ko et al., 2015; USDHHS, 2014). In addition to the individual risks, the problem is compounded since tobacco and cannabis are often used together (El Marroun et al., 2008; Passey et al., 2014; USDHHS, 2014). Approximately one-third of Δ^9-tetrahydrocannabinol (THC), the psychoactive compound in cannabis, crosses the placental barrier (Bailey et al., 1987), and is secreted through breast milk (Perez-Reyes & Wall, 1982). The amount of THC in cannabis has increased substantially over the past two decades (Calvigioni et al., 2014; Mehmedic et al., 2010), making newer studies of developmental outcomes increasingly imperative, especially when combined with comorbid tobacco use.

The prenatal period is a time of enhanced vulnerability during which environmental exposures can have long-lasting effects on child health (Hertz-Picciotto et al., 2008; Wang et al., 2007). Indeed, theoretical models of health such as the fetal origins of health and disease (Barker, 1992; Coe & Lubach, 2008) and the Biological Embedding Model (Miller, Chen & Parker, 2011) highlight the critical role of prenatal and early childhood adversity on children's health outcomes (McEwen & Stellar, 1993; Wadhwa, Buss, Entringer, & Swanson, 2009). Research findings support these theories indicating that prenatal adversity, such as substance exposure, may contribute to a series of developmental processes that amplify risk for poorer health outcomes across the lifespan (Ehrlich, Ross, Chen, & Miller, 2016; Monk, Spicer, & Champagne, 2012). For example, a large literature confirms the developmental consequences of tobacco exposure (Hertz-Picciotto et al., 2008; West, 2002) with findings indicating that prenatal tobacco exposure is associated with a host of poorer health outcomes for children including higher risk for respiratory problems and higher metabolic risk (Ino, 2010; Molnar et al., 2017; Zacharasiewicz, 2016). Further, research indicates that both prenatal and postnatal tobacco exposure affect the maturation of the immune system (Dietert & Zelikoff, 2008). Yet, there are no studies to our knowledge that specifically focus on the effects of cannabis exposure or on the combination of tobacco and cannabis exposures on mucosal immune functioning in young children.

One antibody that plays a critical role in immune functioning of mucus membranes by preventing pathogens from adhering and entering epithelial mucosa is Secretory Immunoglobulin A (SIgA) (Mantis, Rol, & Corthesy, 2011). Immunoglobulin A is one of the major classes of antibodies that protect humans against invading bacteria and viruses, with SIgA found in mucosal secretions such as saliva (Holmgren & Czerkinsky, 2005). SIgA can be identified in saliva very early in life (i.e., often within the first few days) and it tends

to increase across the first 5 years of life (Cole et al., 1999; Seidel et al., 2001). SIgA is a primary defender against infection since the majority of infectious agents first pass into the body through mucosal surfaces (Kaetzel, 2007). For example, findings indicate that SIgA protects individuals against numerous illnesses such as intestinal, respiratory, and urinogenitary infections, along with periodontal disease and caries (Evans et al., 1995). Further, SIgA has been implicated in chronic airways diseases and atopic eczema (Krasteva et al., 2010; Luethviksson et al., 2005). Finally, evidence also indicates that SIgA is sensitive to both acute and chronic stressors (Laurent et al., 2015; Sergerstrom & Miller, 2004; Vermeer, van Ijzendoorn, Groeneveld, & Granger, 2012). Consequently, SIgA may represent a potentially valuable and marginally intrusive way of assessing immune system functioning with respect to infectious disease and stress-related illnesses (Cieslak et al., 2003). Levels of SIgA that are relatively low may reflect a poorly functioning immune system, whereas levels that are relatively high reflect chronic infections and continuing exposure to environmental toxins constituting high microbial pressure (Sandin et al., 2011).

In this study we examined the possibility that pre- and postnatal exposure to cigarettes and cannabis are associated with individual differences in Secretory Immunoglobulin A (SIgA) levels in early childhood. We chose this developmental period because research has demonstrated that levels of SIgA appear to reach the levels expected of an adult by approximately age 5 or 6 years (Kugler, Hess, & Haake, 1992; Sonesson et al., 2011). Given that individual differences in SIgA during this period may be predictive of SigA-related-health issues later in life, early childhood is an important developmental period to study associations between prenatal and postnatal exposures and SIgA levels. In light of the large amount of toxins delivered to the fetus during pregnancy, we expected that salivary SIgA levels would be higher in children who were exposed prenatally, and that continued exposure (postnatal) would also be associated with higher SIgA levels compared to the non-exposed group in light of the expected additive effects and findings indicating postnatal exposure to toxins contributes to poorer immune system functioning.

METHODS

Participants

This study included 50 mother/infant dyads (29 boys; *Mage* = 68.16 months, *SD* = 3.12 months) recruited at their first prenatal appointment in a local area hospital. Infants were prenatally exposed to either cigarette only (n=19), cigarette and cannabis (n=19), or had no prenatal substance exposure (n=12). Pregnant women were screened for eligibility using a self-reported health screening measure, followed by HIPAA consent, and medical record review, with all procedures and consents approved by the IRB. Eligibility criteria included: < 20 weeks gestation, no multiple births, >18 years old, no illicit drug use except cannabis, no heavy alcohol use (4 or more standard drinks on one occasion or >1 drink/day upon pregnancy recognition), and were English speakers. It should be noted that heavy cannabis use was originally an exclusion criteria, but this criteria was relaxed early in the study to aid external validity. At the conclusion of each recruitment month, participating smokers were matched on maternal age and highest educational attainment with the closest eligible

nonsmoking woman, who was then invited to participate. The smoking group was oversampled such that one non-smoker was recruited for every two smokers.

Informed written consent was obtained from interested, eligible women at their first laboratory visit in the first trimester of pregnancy. Prenatal assessments were conducted once in each trimester of pregnancy and at 2, 9, 16, 24, 36, and 60 months of child age. SIgA levels were assayed at the 60 month assessment. The study protocol was approved by the Children and Youth Institutional Review Board at the State University of New York. Participants were informed that data confidentiality was protected by a Federal Certificate of Confidentiality issued by the National Institute on Drug Abuse. Participants received payments for completed assessments at all visits.

Maternal age ranged from 18 to 35 years at the time of their first appointment (M = 22.88 years, SD = 3.58), with 45% African-American, 35% Caucasian, 14% Hispanic, and 12% other, with .04% identifying as more than one race. Forty-six percent of the expectant mothers were married or living with their partner, 36% were in a relationship but not living with their partner, and 18% were single. Twenty-two percent of the women had less than a high school education, 38% completed high school, 26% completed some college, 14% had a vocational/technical or associates degree, and no women had a bachelor's degree or higher.

Of the 50 mothers/infant dyads, five were excluded from the analyses. Three of the infants were excluded due to above normal lead levels (i.e., levels > 5ug/dL), and two infants were excluded because they were sick on the day of the saliva collection. This resulted in a final analytic sample of 45 infants.

Procedures and Instruments

Prenatal Substance Exposure—Assignment to prenatal substance exposure groups (no exposure, cigarette only, or cigarette and cannabis) was based on both self-report and biological validation measures. The Timeline Follow-Back Interview (TLFB; Sobell and Sobell, 1996) was used once at the end of each trimester to gather daily cigarette, cannabis, and alcohol use for the previous three months. The TLFB has good test-retest reliability, and is highly correlated with other intensive self-report measures (Sobell & Sobell, 1996). The TLFB yielded daily data on the number of cigarettes and joints smoked per day as well as the average number of standard alcoholic drinks per day. The no substance exposure group smoked 0 cigarettes and 0 joints per day during each trimester of pregnancy. The cigarette exposure only group smoked between 1 and 15.1 cigarettes per day in trimester one, between 0 and 15.44 cigarettes per day in trimester 2, and between 0 and 17.32 cigarettes per day in trimester 3. They smoked 0 joints per day in all three trimesters. The cigarette and cannabis exposed group smoked between 1.33 and 18.16 cigarettes per day in trimester one, between 0 and 14.05 cigarettes per day in trimester 2, and between 0 and 18.54 cigarettes per day in trimester 3. They smoked between 0 and 5.07 joints per day in trimester 1, between 0 and 2.54 joints per day in trimester two, and between 0 and 2.77 joints in trimester 3 (see Table 1). However, all women in the both group smoked cannabis at some point in their pregnancy.

Maternal saliva specimens were collected once at the end of each trimester and assayed for both cotinine (the primary nicotine biomarker) and THC (the primary cannabinoid biomarker), using enzyme-linked immunosorbent assay (ELISA for the first 42 participants at the first trimester only) or liquid chromatography-tandem mass spectrometry (LC-MSMS) at 10ng/ml cutoff for cotinine and 4mg/ml cutoff for THC. Maternal salivary cotinine ranged from 0 to 547ng/ml of saliva, whereas salivary THC ranged from 0 to 96ng/ml.

Infant meconium, the first neonatal feces, was collected after birth twice daily until the appearance of milk stools. Meconium was transferred to storage containers and frozen at −80°**C** until transport to the National Institute on Drug Abuse. Meconium was assayed with a validated quantitative LS-MSMS method for nicotine, cotinine, and *trans*-3'–hydroxycotinine (OHCOT; see (Gray et al., 2010a) for further details). Limits of quantification were 2.5 ng/g for nicotine, 1ng/g for cotinine, and 5ng/g for OHCOT. As the gold standard for measuring fetal exposure, meconium is a reliable measure of fetal cigarette exposure in the third trimester specifically (Gray et al., 2010b). Additionally, infant meconium was assayed for meconium samples were assayed using a validated 2-dimensional GC-MS method for THC; 11-hydroxy-THC; 8,11-dihydroxy-THC; 11-nor-9-carboxy-THC (THCCOOH); and cannabinol (see Gray et al., 2010b for further details). Limits of quantification were 10ng/g for all analytes, except 11-hydroxy-THC at 15ng/g.

Negative results on all substance use measures resulted in assignment to the no exposure group. A positive result on any measure of cigarette exposure combined with a negative result on all measures of cannabis exposure resulted in assignment to the cigarette only exposure group. Finally, a positive result on any measure of cigarette exposure in combination with a positive result on any measure of cannabis exposure resulted in assignment to the cigarette and cannabis exposure group. The correlations between self-reported use and biological assays ranged from .63 to .64, $p < .01$ for cigarettes and .59 to .87, $p < .01$ for cannabis.

Postnatal Substance Exposure—Postnatal cigarette exposure was measured via child salivary cotinine at 2, 9, 16, 24, and 60 months of child age. Child saliva was assayed for cotinine using ELISA. Child cotinine levels ranged from 0 to 30ng/mL at 2 months, 0.1 to 22.4ng/mL at 9 months, .16 to 29.4ng/mL at 16 months, 0 to 15ng/mL at 24 months, and .06 to 22.46ng/mL at 60 months child age. Postnatal cannabis exposure was assessed via maternal self-report using the TLFB at 2, 9, 16, 24, 36, and 60 months child age. Average cannabis use per day from 2 months to 60 months was computed by calculating the mean across time points. Postnatal cannabis use ranged from 0 to .91 joints/day at the 2 month assessment ($M = 0.04$, $SD = 0.15$), 0 to 6.76 joints/day at the 9 month assessment ($M = 0.30$, $SD = 1.16$), 0 to 2.88 joints/day at the 16 month assessment ($M = 0.13$, $SD = 0.47$), 0 to 4.07 joints/day at the 24 month assessment ($M = 0.23$, $SD = 0.76$), 0 to 4.16 joints/day at the 36 month assessment ($M = 0.24$, $SD = 0.96$), and 0 to 3.33 joints/day at the 60 month assessment ($M = 0.21$, $SD = 0.64$). The average of the combined assessment points was used for each postnatal variable in analyses.

SIgA—Saliva samples were collected from each child at their kindergarten laboratory visit. All samples were taken in the afternoon between 1pm and 5pm to control for time of day

effects (e.g., Vermeer, van Ijzendoorn, Groeneveld, Granger, 2012). Following Granger et al. (2007), a whole saliva sample was collected from each child at their kindergarten laboratory visit by passive drool. Each child was given on average 2 to 3 minutes (but no more than 10 minutes) to donate approximately .5 ml of whole saliva into a 2 mL cryovial. The samples were placed in a refrigeration unit until they were frozen at −80°C that same day. All samples were transported to the Institute for Interdisciplinary Salivary Bioscience were they were stored at – 80 C until the day of assay.

On the day of assay, sample volume (mL) was estimated by weight and SIgA was assayed using a commercially available indirect competitive immunoassay without modification (Laurent, Stroud, Brush, D'Angelo, & Granger, 2015; Vermeer, van Ijzendoorn, Groeneveld, Granger, 2012) to the manufacturers recommended protocol (Salimetrics, Carlsbad, PA). The sample test volume was 25 ul, and calibrator range was 2.5 to 600 ug/mL. Samples were run in duplicate and mean values were calculated for each sample. On average, inter and intra-assay coefficients of variation were less than 15 and 10% respectively. The minimal concentration of SIgA that can be distinguished from 0 is 2.5 μg/mL.

STATISTICAL ANALYSES

Prior to analyses, SIgA measures were inspected for univariate outliers operationally defined as values with a standard deviation greater than 3.29 above the mean. Applying this criterion, no outliers were identified in any of the three groups. Skewnesss and kurtosis levels indicated that SIgA levels were within the acceptable range (Kline, 2011) in all three groups. A univariate analysis of variance was conducted to investigate whether prenatal exposure to cigarette and to both cigarette and cannabis predicted SIgA ug/mL in preschool children. Three groups of children were examined: 1) non-exposed children; 2) children prenatally exposed to cigarette; and 3) children prenatally exposed to both cigarette and cannabis. A priori contrasts comparing the non-exposed group to the cigarette only group and the non-exposed group to the cigarette and cannabis group were of primary interest. Although we report the results of all statistical tests conducted, we largely rely on effect sizes to interpret our findings in light of the relatively small sample sizes for each group.

Second, a hierarchical multiple regression using SPSS version 24 statistical software was conducted to determine whether there were dose-response associations between prenatal exposure and SIgA. An interaction term for amount of maternal cigarettes smoked during pregnancy by the amount of cannabis joints smoked during pregnancy was computed using mean centered variables to test whether the combination of prenatal cigarette and cannabis exposure predicted SIgA ug/mL in preschool children. Further, prenatal cannabis exposure was transformed using the square root function prior to analyses to address non-normality.

Third, a hierarchical multiple regression model was tested such that children's SIgA ug/mL levels were regressed on maternal cigarette and maternal cannabis use during pregnancy on step 1 followed by the interaction term in step 2 of the regression equation. Significant interactions were further probed using a series of post hoc regression equations, referred to as simple slopes analysis (Aiken & West, 1991). Finally, following the procedures outlined above, a hierarchical multiple regression in which SIgA ug/mL levels were regressed on

prenatal and postnatal cigarette and cannabis exposure on step 1, followed by the interaction between postnatal cigarette and cannabis exposure on step 2 of the regression equation. Postnatal maternal cannabis exposure was transformed using the square root function prior to analyses to address non-normality.

RESULTS

Descriptive statistics for all three groups are presented Table 1. Given that the assumption of homogeneity of variance was not met ($F(2, 42) = 7.41$, $p = .002$) we report Welch's adjusted F ratio. Based on Welch's adjusted F ratio, the results of the ANOVA testing whether prenatal exposure to cigarettes and to both cigarettes and cannabis predicted SIgA ug/mL in preschool children was statistically significant Welch's $F(2, 24.76) = 5.84$, $p = .008$ with a moderate effect size ($\omega^2 = .07$) (see Figure 1). The a priori contrast comparing the non-exposed group to the cigarette only group was marginally significant $t(18.27) = 1.98$, $p = .063$, with a large effect size ($d = .93$). The a priori contrast comparing the non-exposed group to the cigarette and cannabis group was statistically significant $t(20.52) = 3.06$, $p = .006$, with a large effect size ($d = 1.35$).[1]

Overall, results indicated that the model regressing SIgA ug/mL levels on prenatal cigarette exposure, cannabis exposure, and their interaction was not statistically significant ($F_{3,41} = .96$, $p = .419$, $R^2 = .07$). Further, amount of maternal cigarette use during pregnancy ($b = .89$, se = 2.79, $\beta = .05$, $p = .751$, 95% CI [−4.73, 6.51]), amount of maternal cannabis use during pregnancy ($b = 42.26$, se = 31.72, $\beta = .21$, $p = .190$, 95% CI [−21.74, 106.27]) and their interaction ($b = -5.89$, se = 8.91, $\beta = -.13$, $p = .476$, 95% CI [−22.43, 10.65]) did not predict SIgA ug/mL among the children, indicating no dose-response associations.[1] Results from the model regressing SIgA ug/mL on postnatal cigarette, postnatal cannabis exposure, and their interaction after accounting for the effects of prenatal cigarette and cannabis exposure indicated that the overall model was not statistically significant ($F_{5,39} = 2.00$, $p = .100$, $R^2 = .20$). Whereas prenatal cigarette ($b = -.74$, se = 3.04, $\beta = -.04$, $p = .809$, 95% CI [−6.89, 5.41]), prenatal cannabis ($b = -22.65$, se = 52.79, $\beta = -.11$, $p = .670$, 95% CI [−129.34, 84.05]), postnatal cigarette ($b = 3.12$, se = 2.95, $\beta = .18$, $p = .296$, 95% CI [−2.84, 9.07]), and postnatal cannabis exposure ($b = 76.68$, se = 51.87, $\beta = .39$, $p = .147$, 95% CI [−28.15, 181.52]) did not predict SIgA ug/mL, the interaction between postnatal cigarette and cannabis exposure was statistically significant ($b = 20.04$, se = 9.79, $\beta = .33$, $p = .047$, 95% CI [.24, 39.83]) (see Figure 2). Results of follow-up simple slopes analyses revealed that a significant positive relationship existed between postnatal cigarette exposure and SIgA ug/mL when postnatal cannabis exposure was relatively high ($b = 12.38$, se = 4.30, $p = .007$, 95% CI [3.66, 21.09]), whereas, there was no association between prenatal cigarette exposure and SIgA ug/mL when postnatal cannabis exposure was relatively low ($b = .94$, se = 3.71, $p = .802$, 95% CI [−6.56, 8.43]).[2]

[1]Analyses are presently reported without the inclusion of potential confounds given the small sample size and considerations of power. However, analyses were also conducted to account for some potential confounds that are typically accounted for when assessing prenatal exposure and immune system functioning such as child sex, race, and medication usage. There were no meaningful differences in results when the effects of child sex, race, and medication usage were accounted for in the analyses.

[2]Analyses are presently reported without the inclusion of potential confounds given the small sample size and considerations of power. However, analyses were also conducted to account for some potential confounds that are typically accounted for when assessing prenatal exposure and immune system functioning such as child sex, race, and medication usage. Further, total number of

DISCUSSION

Few studies have examined the effects of prenatal and postnatal cigarette exposure on SIgA levels among young children and to our knowledge no published studies have examined the effects of the combination of cigarette and cannabis exposure on SIgA levels during early childhood. Results from this prospective longitudinal study indicate that both prenatal cigarette exposure and the combination of prenatal cigarette and cannabis exposure predicted higher levels of SIgA in comparison to a demographically matched control group. We did not find evidence to support dose-response associations. Yet, presence or absence of exposure was predictive of higher levels of SIgA, suggesting that prenatal cigarette exposure and the combination of prenatal cigarette and cannabis exposure alone is sufficient to influence individual differences in mucosal immune functioning.

Our results are in line with those of Noakes et al. (2007) who also found that maternal smoking was associated with higher levels of SIgA levels at 12 months of age and extend their findings by demonstrating that these effects persist into school age. Higher levels of SIgA in the exposed vs non-exposed comparison group may reflect the long-term consequences in the mucosal immune compartment of chronic exposure to the highly immunogenic components of cigarette and cannabis smoke (Sandin, 2011). Meta-analyses of environmental cigarette smoke or postnatal cigarette exposure have noted associations with allergic sensitization in children, particularly those below 7 years of age (Feleszko et al., 2014), with greater risk for emergency or urgent care visits, more wheeze symptoms, and poor lung function (Wang et al., 2015), and physician diagnosed asthma (Tinuoye, Pell & Mackay, 2013). Our results support and extend these findings to indicate higher levels of mucosal immune activation among children not exhibiting clinical symptoms and who were exposed to both prenatal and postnatal cigarette and cannabis.

In addition to cigarette exposure, our results also point to the importance of assessing prenatal cannabis exposure in conjunction with prenatal cigarette exposure given that children prenatally exposed to both substances had the highest levels of SIgA relative to children in our comparison group. Although cannabis is the most widely used drug among pregnant women and that many women who use cigarette while pregnant also use cannabis (El Marroun et al., 2008; Health and Services, 2014; Passey et al., 2014), studies that examine immune function among children prenatally exposed to cannabis or to the combination of cannabis and cigarette are scarce. This is a striking omission from the literature in light of evidence indicating that prenatal cannabis exposure is associated with a host of adverse health-related outcomes including T cell dysfunction and reduced immune response in mice and humans (Lombard et al., 2011; Zumbrun et al., 2015). Our results indicate that the combination of the two substances prenatally results in elevated levels of salivary SIgA relative to the comparison group. Although the effect size for this group difference was large, this result needs to be viewed with caution given the small group sizes and given the results from postnatal exposure, that the prenatal group differences were a

breastfeeding days was also considered with respect to analyses assessing postnatal exposure. There were no meaningful differences in results when the effects of child sex, race, and medication usage were accounted for in the analyses.

reflection of ongoing postnatal exposure since most women who used cigarette and cannabis prenatally also continued postnatally.

Indeed, findings also showed that after accounting for the effects of prenatal cigarette and cannabis exposure, higher levels of postnatal cigarette exposure were associated with higher levels of SIgA when postnatal cannabis exposure was relatively high. However, there was no association between postnatal cigarette exposure and SIgA levels when postnatal cannabis exposure was relatively low. In other words, the combination of exposure to the two substances postnatally was associated with the highest levels of salivary SIgA. Previous studies have found that postnatal cigarette exposure is associated with higher levels of SIgA (Sandin et al., 2011). However, this is the first study to our knowledge that has demonstrated that this association is moderated by postnatal cannabis exposure. Given the small sample size, the results should be viewed as preliminary and suggestive. In light of comorbid use of cigarette and cannabis, the increasing potency of cannabis in recent years, and the changes in legalization that may result in increasing cannabis use among pregnant women, this is an important are for further study with large public health implications.

The findings from this study must be interpreted within the context of its limitations. First, precise measurement of substance use both prenatally and postnatally is difficult. Pregnant and postpartum women are often reluctant to disclose substance use information. However, a significant strength of this study was the utilization of multiple methods to determine prenatal and postnatal substance use, which partially alleviated this limitation.

Second, our results are based on a relatively small sample of young and low-income women. Therefore, in future research studies, larger, more diverse samples would help elucidate these relationships in multiple ways and thus make them more generalizable. Indeed, women who use both cannabis and cigarettes may be qualitatively different from mothers who use just cigarettes. Thus, larger samples will allow for the inclusion of potential confounders that may also account for our findings and further permit the examination of sex-related differences. This sample was also restricted to pregnant smokers or non-smokers with low levels of alcohol use, largely low to moderate marijuana use during pregnancy, and no other illicit substance use during pregnancy. Consequently, whereas our findings are generalizable to the large majority of low-income smokers with lower levels of other substance use, they may not be relevant to pregnant smokers who use other substances.

Third, given that the main goal of the study was to examine cigarette effects, postnatal assessments of cigarette exposure were more thorough than postnatal assessments of cannabis. Most studies of prenatal substance exposure do not use any child biological assays of postnatal exposure. Thus, the inclusion of child postnatal cotinine is a strength, but not having a similar measure for cannabis is a weakness that should be addressed in future studies.

With respect to postnatal exposure we chose to create variables that captured chronic exposures from 2 to 60 months. However, given our small sample size we were unable to determine whether exposures more proximal to the measurement of child SIgA would result in stronger associations. Researchers are encouraged to examine this issue given that it may

have been possible that some mothers abstained from smoking or smoked less while breastfeeding, but then resumed heavier use after weaning, which may have resulted in heavier exposure closer in time to the measurement of child SIgA.

Finally, research supports that salivary flow rate under certain conditions (e.g., when an exogenous source is used to stimulate saliva production during sample collection, like citric acid, sugar, chewing gum) has the potential to influence levels of SIgA. Here, as in Vermeer et al. (2012), our sample collection conditions were controlled, saliva flow was not stimulated, and there is no specific reason to expect between group differences in saliva flow rate. Nevertheless, it is possible that salivary flow rate could have contributed unsystematic error in our SIgA measurements and thus future research examining the role of prenatal and postnatal substance exposure in SIgA should expect that the effect size may be even larger than reported here after accounting for salivary flow rate.

In conclusion, prenatal cigarette and the combination of prenatal cigarette and cannabis exposure were both associated with higher SIgA levels (vs. comparison group) among 5–6 year old children. These findings indicate that the link between prenatal cigarette exposure and SIgA is rather complex, suggesting that we need to move away from linear models examining the association between prenatal cigarette exposure and SIgA to models that examine the joint effects of maternal cigarette and cannabis use. Further studies to differentiate whether the higher levels of SIgA observed in this context represent a hypersensitivity response to cigarette, cannabis, or both would be worthwhile.

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Highlights

- We prospectively examined prenatal and postnatal cigarette and cannabis exposure on mucosal immunity in early childhood.
- Children prenatally exposed to cigarettes and to cigarettes and cannabis had higher Secretory Immunoglobulin A compared to the non-exposed children.
- Children who experienced the combination of postnatal exposure to cigarettes and cannabis had higher levels of SIgA, even after accounting for prenatal exposures.

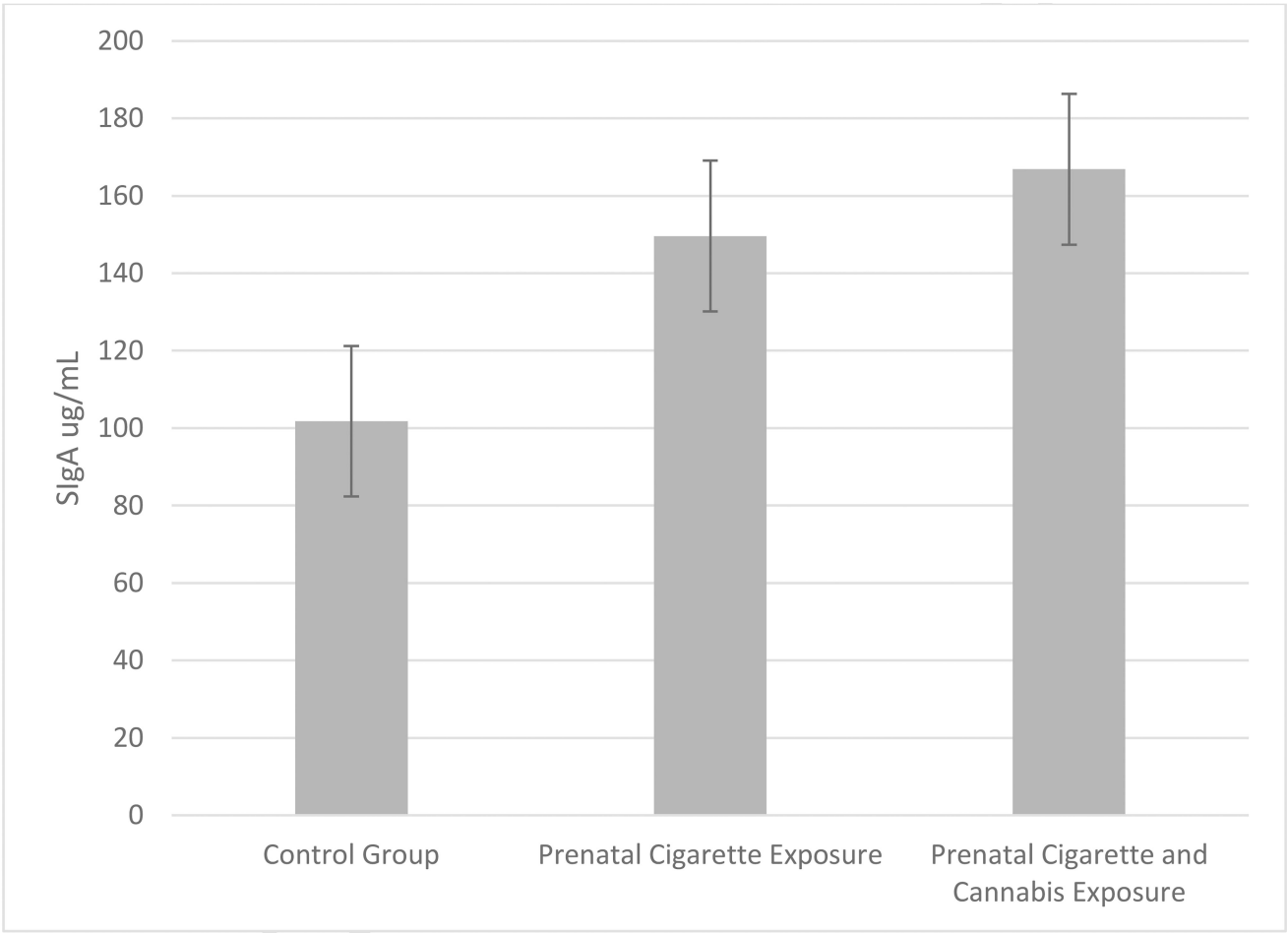

Figure 1.

Prenatal exposure group means with standard errors for SIgA ug/mL. Children in the cigarette only and the cigarette and cannabis groups had higher SIgA levels compared to the non-exposed children

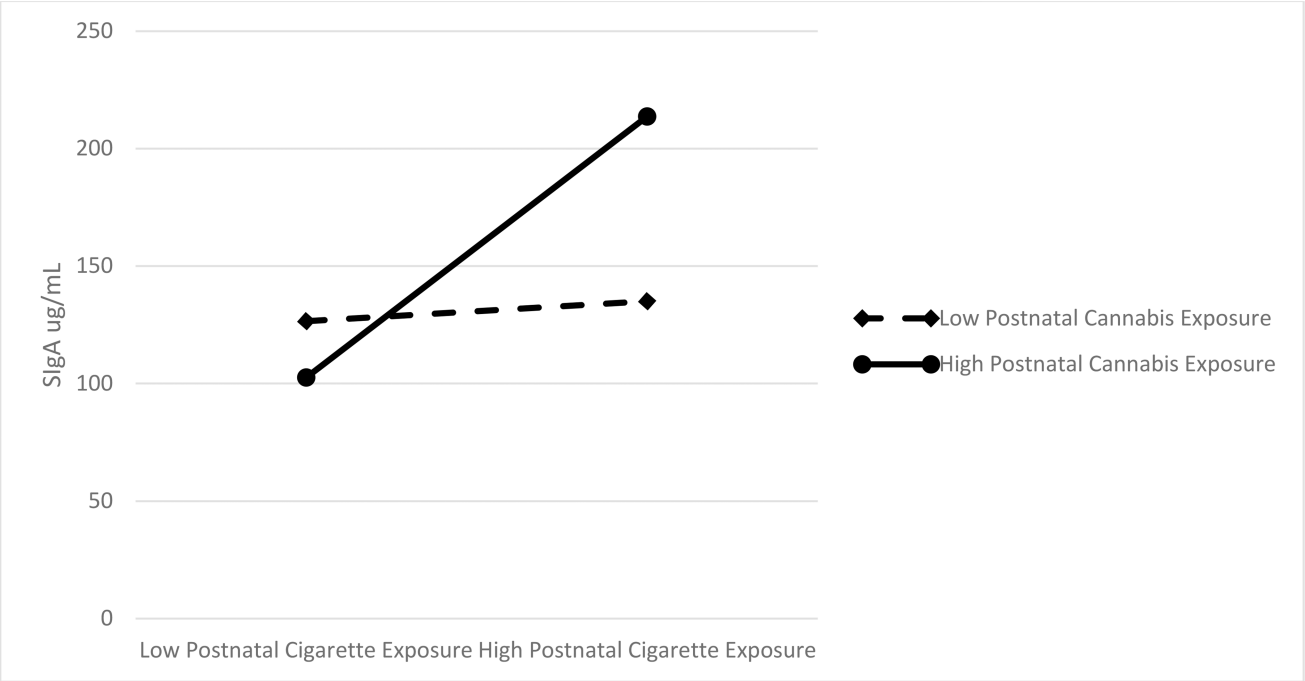

Figure 2.
Two-way interaction between postnatal cigarette and postnatal cannabis exposure on SIgA ug/mL.

Table 1

Descriptive Statistics for Demographic and Substance Use Variables.

Variable	Control Group n = 12		Cigarette Only Group n = 16		Cigarette and Cannabis Group n = 17	
	M	SD	M	SD	M	SD
Maternal Age (years)	20.75	2.60	23.00	3.81	23.35	2.78
Maternal Education (years)	12.50	1.57	12.37	1.78	12.29	1.49
Child Sex (% female)	25.0%	n/a	25.0%	n/a	58.8%	n/a
Medication (% regular medication use)	16.7%	n/a	12.5%	n/a	17.7%	n/a
Race (% Caucasian)	25%	n/a	57.3%	n/a	29.4%	n/a
Total Breastfeeding Days	34.75	48.64	35.25	50.75	43.24	74.99
Average Number of Joints Per Day During Pregnancy	.00	.00	.00	.00	.65	.87
Average Number of Cigarettes Per Day During Pregnancy	.00	.00	4.49	4.53	6.38	4.19
Child Cotinine Levels (ng/ml) 2 months to Kindergarten	1.72	1.53	4.92	4.82	7.66	4.06
Number of Women who Drank Alcohol at Some Point During Pregnancy	6	.n/a	11	n/a	12	n/a
Average Number of Joints Per Day 2 months to Kindergarten	.00	.00	.00	.00	.49	.83
Gestational Age (weeks)	39.50	1.73	38.44	1.86	39.00	1.84
SIgA ug/mL	101.77	26.75	149.60	91.76	166.85	81.88