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Short Communication

Fetal sex is associated with maternal stimulated cytokine production, but not serum cytokine levels, in human pregnancy

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ABSTRACT

Some studies suggest that fetal sex plays a role in maternal physiological processes during pregnancy including glycemic control, blood pressure, and cortisol regulation. However, data examining fetal sex-specific differences in maternal immune parameters is lacking. In the current study, serum levels of interleukin(IL)-6, IL-8, and tumor necrosis factor(TNF)-α as well as LPS-stimulated production of IL-6, IL-8, TNF-α, and IL-1β by PBMCs incubated for 24 h were assessed in early, mid, and late pregnancy among 80 women (46 with male and 34 with female fetuses). Linear mixed models showed that women carrying females versus males exhibited greater stimulated production of IL-6 at each timepoint ($ps \leqslant 0.03$), TNF-α in early pregnancy ($p = 0.04$), and IL-1β in mid- and late pregnancy ($ps \leqslant 0.05$). Despite changes in serum levels of IL-8 ($p = 0.002$) and TNF-α ($p < 0.0001$) across pregnancy, no differences in any serum cytokines were observed in relation to fetal sex ($ps > 0.85$). In conclusion, in pregnant women, those carrying female versus male fetuses exhibited greater stimulated cytokine production across pregnancy. Differential inflammatory responses could affect maternal health and fetal development. Fetal sex should be considered as a factor in studies of maternal inflammation. These findings have relevance both clinically and conceptually. For example, maternal asthma is exacerbated among women carrying female versus male fetuses. In addition, data on associations between fetal sex and maternal immune function among women with health conditions (e.g., preeclampsia) and adverse pregnancy outcomes (e.g., preterm birth) would be informative.

1. Introduction

Pregnancy is characterized by substantial changes in maternal immune parameters. Pregnant women exhibit heightened serum levels of certain proinflammatory cytokines as well as greater lipopolysaccharide (LPS) stimulated proinflammatory cytokine production than non-pregnant adults (e.g., Brewster et al., 2008; Vassiliadis et al., 1998). Although limited, longitudinal examinations have demonstrated significant increases in some serum and LPS-stimulated cytokines across pregnancy, including serum TNF-α, stimulated IL-6, and stimulated IL-1β, while other markers exhibit a decline, such as serum IL-8 (Christian and Porter, 2014; Gillespie et al., 2016). Information regarding factors which may modify such adaptation is limited.

Bidirectional communication between the pregnant woman and fetus has been supported, with fetal sex shown to play a role (Glynn and Sandman, 2011). Specifically, though not found in all studies, some studies have shown that fetal sex is associated with differences in maternal physiology, including cortisol regulation, glycemic control, and blood pressure (DiPietro et al., 2011; Giesbrecht et al., 2015; Hocher et al., 2009; Petry et al., 2007). Studies examining associations between fetal sex and maternal immune parameters are limited. However, some studies have shown differences by fetal sex in immune-relevant gene expression as well as cytokine expression in the placenta, with women carrying female fetuses compared to males showing elevated levels (Clifton and Murphy, 2004; Scott et al., 2009). Data examining whether fetal sex is associated with distinct patterns in maternal inflammatory processes as measured in peripheral blood is lacking.

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Of clinical importance, differences in maternal physiological responses to health conditions (e.g., asthma) have been observed in relation to fetal sex. A substantial body of literature examining asthma in pregnant women shows exacerbated symptom severity in those carrying female fetuses versus male (Clifton, 2010; Clifton and Murphy, 2004; Scott et al., 2009). In addition, asthma is characterized by chronic inflammation, including increased expression of proinflammatory cytokines, such as TNF-α and IL-1β (Kips, 2001). Thus, data examining effects of fetal sex on healthy maternal immune adaptation would be informative.

Addressing gaps in the current literature, the current study examined serum levels of the proinflammatory cytokines interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α as well as LPS-stimulated production of IL-6, IL-8, TNFα, and IL-1β among 80 pregnant women assessed in early, mid, and late pregnancy. This study included 46 women carrying male fetuses, and 34 carrying females. The association between fetal sex and maternal levels of both serum and stimulated cytokine production was examined.

2. Materials and methods

2.1. Study design

Eighty-two pregnant women were recruited from the Ohio State University Wexner Medical Center (OSUWMC) Prenatal Clinic and surrounding community of Columbus, Ohio. Study visits were conducted during early (mean = 12.33, SD = 1.52), mid (mean = 20.61, SD = 1.29), and late pregnancy (mean = 29.22, SD = 1.41). A blood sample was collected at each visit. The broader study captured psychosocial functioning; these data were not utilized in the current analyses. In the current analyses, two women were excluded due to unavailable medical records following delivery resulting in a final sample of 80.

2.2. Participants

Women were ineligible if they had any major immunological conditions (e.g., rheumatoid arthritis), fetal anomaly, illicit drug use after pregnancy was known, or consumed more than two alcoholic drinks per week during pregnancy (per self-report or medical record). Women who described experiencing acute illness (e.g., flu-like symptoms) within 10 days of a study visit were rescheduled. Written informed consent was obtained at the first study visit, and participants received modest financial compensation at the completion of each visit. The study was approved by the OSU Biomedical Institutional Review Board.

2.3. Measures

2.3.1. Maternal demographics and fetal sex

Race/ethnicity, age, marital status, education level, annual household income, current cigarette use, and number of prior births (parity) were collected by self-report at the first study visit. Pre-pregnancy body mass index (BMI; kg/m2) was calculated using self-reported pre-pregnancy weight and measured height at the first visit. Gestational weight gain was calculated using measured weight prior to delivery (per medical record review) and self-reported pre-pregnancy weight. Adverse outcomes (i.e., gestational hypertension, preeclampsia, gestational diabetes, low birth weight, and preterm birth) and fetal sex were obtained per medical record review or self-report.

2.3.2. Serum cytokine levels

Whole blood was collected into vacutainer tubes while participants were in a seated position. Samples were immediately centrifuged, aliquoted, and placed in −80 °C freezer storage until analysis. Serum levels of IL-6, TNF-α, and IL-8 were assayed in duplicate on either single spot ultra-sensitive or multiplex V-Plex kits from Meso Scale Discovery (MSD, Meso Scale Discovery, 1601 Research Blvd, Rockville, MD). Plates were read by an MSD SECTOR Imager 2400 measuring electrochemiluminescence. Sample concentrations were extrapolated from a standard curve calculated using a four parameter logistic fit using MSD Workbench 3.0 software. The limits of detection were 0.31 pg/mL for IL-6, 0.17 pg/mL for TNF-α, and 0.27 pg/mL for IL-8. The inter- and intra- assay coefficients of variation were 8.69% and 5.89% for IL-6, 5.12% and 5.34% for TNF-α, and 5.27% and 3.71% for IL-8, respectively.

2.3.3. Stimulated cytokine production

PBMCs at a concentration of 1×10^6 cells/ml were stimulated with 1ug/ml LPS in RPMI-1640 supplemented with 10% human male serum for 24 h. A non-LPS media control was incubated simultaneously. After 24 h, samples were centrifuged and aliquots removed and frozen at −80 °C until assayed. Media samples were assayed neat, while LPS samples were diluted 1:6. Samples were assayed in duplicate for IL-6, TNF-α, IL-1β, and IL-8 (pg/ml) using human ProInflammatory II multiplex tissue culture kits from Meso Scale Discovery (MSD; 1601 Research Blvd., Rockville, MD). Plates were read by an MSD Sector Imager 2400 measuring electrochemiluminescence. The inter- and intra- assay coefficients of variation were 8.28% and 3.20% for IL-6, 6.02% and 2.36% for TNF-α, 8.59% and 1.91% for IL-1β, and 9.23% and 2.93% for IL-8, respectively.

2.4. Statistical analyses

All analyses were conducted in SAS 9.4. Missing data were addressed utilizing the restricted maximum likelihood estimation method. Serum cytokines were log-transformed (base 10) to fit normality assumptions. Thirty-two data points of serum cytokines ($n = 21$) or LPS-stimulated cytokines ($n = 11$) were classified (±3 SD from mean) as outliers and excluded from respective analyses. To compare demographic characteristics between women who gave birth to girls vs boys, chi-square tests and t-tests were conducted, as appropriate. Mixed effects regression models were used to examine whether maternal serum cytokine levels and LPS-stimulated cytokine production by maternal PBMCs differed by fetal sex. All models included timepoint as an ordinal variable. A subject-level random effect was included in each of the models to account for the dependency between the repeated measures. A risk factor modeling approach was used to examine whether any covariates served as meaningful confounders in the relationship between fetal sex and maternal immune parameters.

3. Results

3.1. Maternal demographics by fetal sex

Participant characteristics for the total sample and by fetal sex are reported in Table 1. No significant differences in age, race, marital status, education, annual income, parity, BMI, gestational weight gain, cigarette use, or adverse outcomes were observed by fetal sex. Age, race, income, BMI, gestational weight gain, and cigarette use did not change coefficient estimate of fetal sex by greater than 15% and thus, consistent with a risk factor modeling approach (Bursac et al., 2008), these were not included in final analyses.