Pregnancy Hypertension 12 (2018) 53–57

Contents lists available at ScienceDirect

Pregnancy Hypertension

journal homepage: www.elsevier.com/locate/preghy

The impact of female fetal sex on preeclampsia and the maternal immune milieu

Brandie D. Taylor[a,*], Roberta B. Ness[b], Mark A. Klebanoff[c], Gong Tang[d], James M. Roberts[e,f], David M. Hougaard[g], Kristin Skogstrand[g], Catherine L. Haggerty[f]

[a] Department of Epidemiology and Biostatistics, Texas A&M University, School of Public Health, College Station, TX, USA
[b] University of Texas School of Public Health, Houston, TX, USA
[c] The Research Institute at Nationwide Children's Hospital, Columbus, OH, USA
[d] Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA
[e] Magee-Womens Research Institute, Department of Obstetrics and Gynecology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA
[f] Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA
[g] Danish Centre for Neonatal Screening, Department of Congenital Disorders, Statens Serum Institut, Copenhagen, Denmark

ARTICLE INFO

Keywords:
Fetal
Inflammation
Preeclampsia
Postpartum

ABSTRACT

Objective: Small studies suggest that fetal sex alters maternal inflammation. We examined the association between fetal sex, preeclampsia and circulating maternal immune markers.

Methods: This was a secondary data analysis within a nested case-control study of 216 preeclamptic women and 432 randomly selected normotensive controls from the Collaborative Perinatal Project. All women had singleton, primiparous pregnancies without chronic health conditions. Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for associations between female fetal sex and preeclampsia. Outcomes included preeclampsia, preterm preeclampsia (< 37 and < 34 weeks), and normotensive preterm birth < 37 weeks. Associations between female fetal sex and immune markers [interleukin (IL)-6, IL4, IL5, IL12, IL10, IL8, IL1-beta, interferon (IFN)-gamma, tumor necrosis factor (TNF)-beta, and transforming growth factor-beta] were examined using a statistical method developed for large proportions of censored biomarker data. Models were adjusted for maternal age, race, body mass index, and smoking.

Results: Women with early preterm preeclampsia (< 34 weeks) had higher odds of having a female fetus ($OR_{adj.}$ 3.2, 95% CI 1.1–9.6) and women with normotensive preterm birth had lower odds ($OR_{adj.}$ 0.5, 95% CI 0.3–0.9). Female fetal sex was associated with lower first trimester pro-inflammatory IFNγ and IL-12 but higher second trimester pro-inflammatory IL1β and TNFβ, anti-inflammatory IL4r, and regulatory cytokines IL5 and IL10. Female fetal sex was associated with higher postpartum IL10 in preeclamptic women only.

Conclusions: We identified sexual dimorphism in maternal inflammation. Longitudinal studies are needed to determine if fetal sex impacts the maternal immune milieu across pregnancy.

1. Introduction

Sexual dimorphism is present in several pregnancy complications. Observational studies consistently report associations between male fetal sex and preterm birth, fetal loss, and infant mortality [1–3]. A meta-analysis found that male fetal sex increased the risk of pre-eclampsia/eclampsia (relative risk = 1.1) in non-Asian populations [4]. Another meta-analysis reports that female fetal sex is associated with preterm preeclampsia < 37 weeks (odds ratio 1.1) and < 34 weeks (odds ratio 1.4) of gestation [5]. Thus, the literature remains conflicting.

Despite evidence that sexual dimorphism may influence pregnancy outcomes the mechanisms are not elucidated. In a study of 80 women, stimulated production of interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL1β, but not circulating cytokines, were higher in women with female fetuses [6]. In 38 healthy women, women with female fetuses had higher serum regulatory cytokines (IL-5, IL-9, IL-17, and IL-25) while women with male fetuses had higher pro-inflammatory markers (G-CSF, IL-12p70, IL-21, and IL-33) across pregnancy [7]. Clifton et al. reports sexual dimorphism in placental adaptation. Female placentas have significant changes (e.g. immune gene expression) in response to maternal asthma while male placentas display little change

* Corresponding author at: Department of Epidemiology and Biostatistics, School of Public Health, Texas A&M University, 228 SPH Administration Building, TAMU 1266, College Station, TX 77843-1877, USA.
E-mail address: Taylor@sph.tamhsc.edu (B.D. Taylor).

https://doi.org/10.1016/j.preghy.2018.02.009
Received 15 August 2017; Received in revised form 20 February 2018; Accepted 23 February 2018
Available online 24 February 2018

[8,9].

The hypothesis that fetal sex affects the maternal immune milieu is interesting and aligned with sex differences in immune function across the life-course (e.g. males are susceptible to some infectious diseases and females are biased towards auto-immunity) [10]. However, investigations of fetal sex, maternal immunity and pregnancy outcomes are limited to a handful of small studies. Only one study has examined fetal sex and maternal immune markers postpartum [7], finding no differences. Exploring the postpartum period among complicated pregnancies may be warranted as preeclampsia increases the risk of cardiovascular disease by 3-fold [11]. Additionally, maternal immune markers differ by race/ethnicity and future investigations should account for this potential confounding factor [12].

This study examined associations between fetal sex, preeclampsia and the systemic maternal immune milieu during pregnancy and postpartum. We utilized a sandwich immunoassay which is appealing for large investigations as they are cost saving, measure multiple biomarkers and required smaller sample volume. However, immunoassays rely on identifying epitopes which can be blocked by interactions between cytokines and host blood proteins [13] resulting in subjects measuring outside of the limit of detection (LOD) [14]. To increase the validity of our analysis to handle data below the LOD, we utilized a statistical method developed for large proportions of censored biomarker data [15].

2. Methods

2.1. Population and study design

This was a secondary data analysis of a nested case control study within the Collaborative Perinatal Project (CPP), a longitudinal study of 55,908 pregnancies [16] enrolled between 1959 and 1965 from 12 university-affiliated medical centers in the United States. Oral consent (standard at the time) was obtained [17]. The nested case control study examined associations between immune biomarkers and preeclampsia [18] and included a random sample of 216 preeclamptic who had primiparous and singleton pregnancies. Exclusions included a history of diabetes, cardiovascular disease or hypertension or no stored first study visit serum samples (collected prior to 27 weeks of gestation with no recorded thaws). Two normotensive controls (1:2) were randomly selected for each case using the above inclusion/exclusion criteria. Matching was not used in this study. The Texas A&M University Institutional Review Board approved the current investigation.

2.2. Outcome measurements

Preeclampsia was the primary outcome and in the CPP was based on chart abstraction of blood pressure and protein levels and defined as new gestational hypertension after 20 weeks of gestation (≥two measurements of systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) and proteinuria (two random urine dipsticks of 1 + protein or one dipstick of 2 + protein). In the intrapartum period, the first five pressures obtained after hospital admission for delivery were averaged.

Secondary outcomes were included in this study. It is accepted that preeclampsia is a heterogeneous disease with subtypes that have different pathophysiological pathways [19,20]. Preeclampsia can be classified as term preeclampsia (birth ≥37 weeks gestation) or preterm preeclampsia (birth < 37 weeks gestation or < 34 weeks gestation). Cases of preeclampsia can also be considered severe if they had at least one of the following symptoms: systolic blood pressure ≥160 mmHg, diastolic blood pressure ≥110 mmHg, proteinuria of 5 g/24 h, proteinuria of 3+ or more, oliguria, pulmonary edema, or convulsions/eclampsia. The HELLP syndrome had not yet been described at the time of the CPP, and liver function tests and platelet counts were not included in the database. Small for gestational age (SGA) was defined previously in the CPP as a birth weight < 10th percentile for gender, race, and gestational age using birth weight distributions. As fetal sex is implicated in preterm birth, we examined preterm birth < 37 weeks of gestation in normotensive controls only. Gestational age was determined by the date of delivery minus the date of last menstrual period.

2.3. Cytokine measurements

In the CPP, first study visit fasting blood samples were collected in glass vacutainers. After separation, maternal serum was stored at −20 °C. Serum samples were monitored continuously from the time of collection and had no recorded thaws. Immune markers were measured in duplicate with an in-house multiplex flow cytometric assay system (LabMap, Luminex Corporation, Austin, Texas) at the Statens Serum Institute in Copenhagen [13]. Calibration curves for analytes were calculated by the Bio-Plex 3.0 software (BioRad, US). Mean intra- and inter-assay CVs were 6.2% and 16%, and ranged from 6.7 to 13 (IL4 and TNF-α) and 10 to 25 (IL-4 and TNF-α) [13]. We acknowledge that the long-term storage of CPP samples raises concerns. Our assay was validated using ten anonymously collected residual dried blood spot specimens stored for 23 years at −24 °C in the Danish biological bio-bank [13]. In a CPP study, cytokines were measured and compared to fresh samples finding consistent results [21]. Furthermore, the same immune markers measured using CPP samples had similar proportions of non-detectable levels compared to the more contemporary Danish National Birth Cohort (DNBC) [22,23]. As IL2, IL1α, and TNF-α measured below the LOD in 75% of patients, they were excluded. Table 1 shows the median, interquartile range and proportion below the limit of detection for each marker. Associations between immune markers and preeclampsia in the CPP have been described previously [24].

Table 1
Medians, interquartile range (IQR) and proportion below the limit of detection (LOD) for each serum immune marker.

Immune marker	First Trimester N = 250 Median (IQR) % below LOD	Second Trimester N = 392 Median (IQR) % below LOD	Postpartum N = 591 Median (IQR) % below LOD
IFNγ pg/ml	4 (6)	4 (4)	4 (0)
	50% < LOD	50% < LOD	75% < LOD
IL1β pg/ml	9 (12)	10 (13)	9 (13)
	25% < LOD	25% < LOD	25% < LOD
IL4 pg/ml	21.5 (70)	20 (71)	18 (67)
	25% < LOD	25% < LOD	25% < LOD
IL4r pg/ml	239.5 (327)	250 (302)	198 (278)
	10% < LOD	5% < LOD	5% < LOD
IL5 pg/ml	13 (16)	11 (15)	12 (18)
	25% < LOD	25% < LOD	25% < LOD
IL8 pg/ml	19 (101)	18 (113)	4 (18)
	25% < LOD	25% < LOD	50% < LOD
IL6 pg/ml	73.5 (197)	4 (209)	96 (229)
	10% < LOD	25% < LOD	10% < LOD
IL10 pg/ml	42 (86)	43 (97)	18 (109)
	25% < LOD	25% < LOD	10% < LOD
IL12 pg/ml	4 (11)	4 (10)	4 (9)
	50% < LOD	50% < LOD	50% < LOD
MIF pg/ml	16.9 (28.5)	17.4 (32.9)	23 (42)
	10% < LOD	10% < LOD	5% < LOD
MIP pg/ml	193 (259)	201 (283)	195 (272)
	10% < LOD	10% < LOD	10% < LOD
TGFβ pg/ml	665 (1030)	225 (1083)	706 (1148)
	10% < LOD	10% < LOD	10% < LOD
TNFβ pg/ml	10 (20)	10 (15)	10 (0)
	50% < LOD	50% < LOD	75% < LOD
TNFr pg/ml	0.52 (0.59)	0.52 (53)	0.96 (0.95)
	10% < LOD	5% < LOD	5% < LOD