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Short communication

Preeclampsia is associated with an increased pro-inflammatory profile in newborns

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ABSTRACT

Hypertensive disorders of pregnancy (HDP) lead to high rates of maternal and fetal morbidity. Existing studies on inflammatory marker TNFα in HDP offspring are inconsistent. We performed a population-based cohort study of 636 pregnancies, including normotensive (NT) women and women with preeclampsia (PE) or gestational hypertension (GH). TNFα was measured in maternal blood in the first and second trimesters and in cord blood at the time of delivery. Cord blood TNFα was higher in offspring delivered of women with PE (6.53 [4.94–8.38]pg/mL) versus those delivered of NT women (5.13 [4.11–6.72]pg/mL; $p = 0.01$), independent of confounders. Maternal TNFα levels were not different among groups ($p > 0.1$) in either the first or second trimester.

1. Introduction

Preeclampsia (PE) is a common obstetric complication affecting up to 10% of pregnancies worldwide (Magee et al., 2014). PE, classically defined as new-onset hypertension and proteinuria during pregnancy, is a leading cause of morbidity and mortality of both mothers and offspring (Magee et al., 2014).

Although a careful balance of anti- and pro-inflammatory cytokines is required for a successful pregnancy, evidence is accumulating that PE is characterized by an increased maternal pro-inflammatory response during the third trimester (Xie et al., 2011). TNFα is a well-characterized pro-inflammatory marker in preeclampsia, as measured in maternal blood (Xie et al., 2011). There are far fewer studies on TNFα as a marker of feto-placental pro-inflammatory imbalance, and these have generated conflicting results (Catarino et al., 2012; Tosun et al., 2010; Kupferminc et al., 1999). Thus, we measured TNFα in maternal and cord blood collected in mother–child pairs of women with PE or gestational hypertension (GH) and in normotensive (NT) control women in a large prospective population-based cohort.

2. Materials and methods

2.1. Participants

We recruited pregnant women aged ≥18 years in a prospective population-based observational study during a routine prenatal visit (visit 1 [V1], ≤16 weeks of gestation) at the Blood Sampling in Pregnancy Clinic of the Centre Hospitalier Universitaire de Sherbrooke (CHUS). Exclusion criteria were: pregestational diabetes (type 1 or 2), diabetes diagnosed at V1, presence of antihypertensive therapy or blood pressure (BP) ≥140/90 mmHg at V1, multiple pregnancy, drug and/or alcohol abuse, and any major med-

Abbreviations: BMI, body mass index; BP, blood pressure; CHUS, Centre Hospitalier Universitaire de Sherbrooke; GH, gestational hypertension; Hb_{A1c}, glycated hemoglobin; HDP, hypertensive disorders of pregnancy; NT, normotensive; PE, preeclampsia; V1, visit 1; V2, visit 2.

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ical conditions. Mothers with chronic inflammatory conditions and chronic systemic steroidal medication use, or with acute infection at any visit, and mother–child pairs without cord blood collection were excluded from the following analyses. The CHUS ethics board approved the study protocol, and every participant gave written informed consent before enrollment in the study, in accordance with the Declaration of Helsinki.

2.2. Classification

Women were classified as having PE if they had gestational hypertension (at least two BP measures ≥140/90 at least 20 min apart, from the 20th week of gestation to 6 weeks after delivery) with proteinuria (≥300 mg/day or protein/creatinine ratio ≥0.3), as per the classic definition (Magee et al., 2014). All cases were retrospectively reviewed and discussed with a nephrology fellow to ensure correct classification based on the presence of hypertension and proteinuria. Women classified as having GH had hypertension without proteinuria during pregnancy or up to six weeks after delivery. Women not meeting any of above-mentioned criteria from the first trimester to six weeks post-delivery were classified as being NT. Research data collected at V1 and again at 24–28 weeks (visit 2 [V2]) showed that our participants were indeed normotensive at the beginning of pregnancy. Gestational diabetes mellitus (GDM) status was established according to international criteria (Metzger et al., 2010).

2.3. Measurements

Clinical and anthropometric parameters were measured according to standard research protocols as we previously reported (Guillemette et al., 2014). BP was measured thrice in the sitting position after a rest of at least five minutes, and the mean value was used in all analyses. Questionnaires were used to assess medical history and lifestyle (physical activity and diet) in the previous three months, in addition to the exclusion criteria mentioned above. Anthropometrics, BP, and questionnaire assessments were repeated at V2. Clinical data pertaining to the end of pregnancy and delivery (including BP measurements and proteinuria results) were extracted from electronic hospital data records.

2.4. Blood samples processing

Maternal blood samples were drawn at V1 (non-fasting) and at V2 (fasting). Umbilical cord blood was drawn within 30 min of delivery. Blood samples were immediately centrifuged at 2500 × *g* for ten minutes at 4 °C. Aliquots of plasma were stored at −80 °C until measurement.

2.5. Laboratory measurements

Plasma glucose concentration was measured using the glucose hexokinase method (Roche Diagnostics). Hb_{A1c} was measured by high-performance liquid chromatography (VARIANT; Bio-Rad). TNFα concentration was measured using a multiplexed particle-based flow cytometric assay (Milliplex map kits, Millipore). Intra- and inter-assay coefficients of variation were 3% and 6%; minimum detectable concentration was 0.3 pg/mL, and lowest standard was 0.90 pg/mL.

2.6. Statistical analyses

Values are presented as a percentage for categorical variables and as median and interquartile range for continuous variables. We first compared PE, GH, and NT in the overall cohort. We used the Chi-squared test to compare categorical variables and the Kruskal–Wallis and Mann–Whitney tests for continuous variables to detect differences between groups. We used Spearman's to test correlations, and Friedman's to detect a difference in time between V1 and V2 measures. In addition to cohort-based analyses, a nested case–control allowed comparison of cord blood TNFα levels while controlling for gestational age at delivery and third-trimester maternal BMI, with two NT controls matched to each PE case. A mixed model was used with a random intercept for each matched triad (within-subject factor), fit with exposure group (PE vs NT) as the between-subject factor, and using compound symmetry as a covariance structure, without additional covariates. We considered an α level of 0.05 to indicate statistical significance. Data were analyzed using PASW Statistics 18 (IBM).

3. Results

3.1. Maternal characteristics

We included 636 mother–newborn pairs in our main analyses, including 18 cases with PE and 25 with GH, for an overall 6.8% incidence rate of HDP. Table 1 shows the main characteristics of all participants. Median systolic and diastolic BP were significantly higher in PE and GH mothers at both visits. The three groups were comparable in age, ethnicity, smoking status, and gestational age at both V1 and V2. However, as expected, PE and GH mothers were more likely to be primiparous and heavier than NT mothers. Mothers with GH showed a statistically significant increase in TNFα levels from V1 to V2 (from 1.26 [1.11–1.72] to 1.70 [1.60–2.31]; $p = 0.001$); this was not the case in NT or PE women.

3.2. Cord blood TNFα and birth outcomes

Cord blood TNFα was higher in offspring of women with PE (6.53 [4.94–8.38] pg/mL) versus NT (5.13 [4.11–6.72] pg/mL; $p = 0.02$). We also noted that PE neonates were born earlier than GH and NT neonates, which was probably driven by clinical decisions related to PE diagnosis (induction and C-section). Consequently, we conducted a sensitivity analysis based on a nested case–control (1:2) design, matching for gestational age at delivery and maternal BMI. This mixed model showed that TNFα levels were elevated in cord blood from 18 PE cases vs 36 NT controls (6.53 [4.94–8.38] vs 4.85 [3.56–7.06] pg/mL, $p = 0.01$). We thus conclude that higher inflammation in newborns exposed to PE was not driven by prematurity and was independent of maternal BMI (which was higher in PE cases). Delivery mode and time spent in labor (for vaginal and induced deliveries) were not associated with TNFα levels (p values > 0.05).

4. Discussion

We have demonstrated that offspring born from mothers with PE have higher levels of circulating TNFα at birth compared with NT women, independently of gestational age and maternal weight. Only three studies investigated the impact of PE on the levels of TNFα in newborns and overall showed conflicting results. Higher cord blood TNFα levels after exposure to PE were also found in one study (Tosun et al., 2010), while one study showed no difference between groups (Catarino et al., 2012) and another found lower TNFα levels in PE newborns (Kupferminc et al., 1999). In an attempt to explain these differences, we note that our ELISA assay has both more sensitivity and less intra- and inter-assay variation than those used in the above-mentioned studies (Tosun et al.: 0.7 pg/mL, 6.3% and <10%; Catarino et al.: 5.5 pg/mL, 4.2%, and 4.6%; Kupferminc et al.: 5.0 pg/mL, 8.3% and 9.3% respectively). Greater reliability may have contributed to our ability to detect a significant difference.

Table 1
Characteristics of women and neonates in the normotension (NT), gestational hypertension (GH), and preeclampsia (PE) groups.

	NT ($n=593$)	GH ($n=25$)	PE ($n=18$)	p^*
Median [Q1–Q3] or%				
Maternal baseline characteristics				
Age (years)	28 [26–31]	27 [26–30]	27 [24–32]	0.29
Parity (% primiparous)	33.1	60	55.6	0.004
Ethnicity (% Caucasian)	96.9	96	100	0.72
Smoker (% yes)	8.9	4.0	5.6	0.82
Pregestational BMI (kg/m^2)	23.3 [20.9–27.3]	25.5 [21.4–31.9]	25.4 [23.4–32.5]	0.02
Maternal characteristics at first trimester				
Gestational age (weeks)	9.3 [8.1–11.3]	9.2 [7.6–12.1]	9.4 [7.9–11.3]	0.99
BMI (kg/m^2)	24.0 [21.6–28.1]	26.6 [22.1–32.9]	26.6 [24.7–33.1]	0.01
SBP/DBP (mmHg)	110/68 ± 7/6	120/76 ± 8/5	121/79 ± 6/6	<0.0001
Hb_{A1c} (mmol/L)	5.2 [5.1–5.4]	5.3 [5.1–5.6]	5.2 [5.1–5.3]	0.68
TNFα levels (pg/mL)	1.54 [1.19–2.08]	1.26 [1.11–1.72]	1.61 [1.27–1.99]	0.19
Maternal characteristics at second trimester				
Gestational age (weeks)	26.2 [25.6–27.0]	26.2 [25.6–27.2]	26.0 [25.5–27.1]	0.7
BMI (kg/m^2)	26.7 [24.2–30.5]	28.8 [25.2–34.7]	29.6 [26.3–34.7]	0.02
Weight gain between first and second trimester (kg)	6.7 [4.8–8.3]	7.0 [4.1–8.8]	6.1 [3.5–9.7]	0.65
SBP/DBP (mmHg)	106/67 ± 6/5	117/74 ± 7/6	115/78 ± 8/5	<0.0001
Glycemia during 75 g OGTT (mmol/L)				
Fasting	4.1 [3.9–4.4]	4.2 [4.1–4.6]	4.3 [4.1–4.6]	0.08
1 h	6.9 [5.9–8.0]	7.2 [5.7–8.7]	7.3 [5.8–8.3]	0.95
2 h	5.7 [4.8–6.5]	5.8 [5.0–6.2]	5.7 [5.1–6.6]	0.75
GDM diagnosis (%)	7.3	12.0	11.1	0.57
Fasting TNFα levels (pg/mL)	1.58 [1.15–2.14]	1.70 [1.60–2.31]	1.71 [1.25–2.04]	0.11
ΔTNFα levels between first and second trimester (pg/mL)	−0.01[−0.49–0.58]	0.60 [0.06–0.82]	0.08 [−0.79–0.72]	0.002
Delivery				
Gestational age (weeks)	39.5 [38.6–40.3]	39.4 [38.4–40.3]	37.6 [37.2–39.4]	0.004
Mode				<0.0001
Spontaneous, vaginal (%)	57.8	28.0	5.6	
Induction, vaginal (%)	27.3	64.0	77.8	
Cesarean (%)	14.8	8.0	16.7	
Neonate characteristics				
Neonate weight (g)	3425 [3145–3720]	3285 [2930–3585]	3035 [2770–3394]	0.004
Neonate length (cm)	51.0 [50.0–52.0]	51.0 [49.3–53.0]	49.5 [48.0–53.0]	0.15
APGAR score at 1 min	9 [8–9]	8 [8–9]	9 [6–9]	0.42
Birth weight for gestational age z scores	0.03 [−0.57–0.55]	−0.00[−0.76–0.31]	−0.15[−0.63–0.10]	0.33
Cord blood TNFα levels (pg/mL)	5.13 [4.11–6.72]	5.44 [3.94–6.68]	6.53 [4.94–8.38]	0.05

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; Hb_{A1c}: glycated hemoglobin; Q1: first quartile; Q3: third quartile; OGTT: oral glucose tolerance test; GDM: gestational diabetes mellitus.

* Comparison between groups was made Kruskal–Wallis test continuous variables and Chi-squared test for categorical variables.

To our knowledge, no studies have reported on TNFα changes throughout pregnancy specifically in GH women. Available longitudinal data pertain only to PE. One study found that women who later developed PE had an increase in TNFα levels from the first trimester to delivery, although TNFα remained lower than in NT women (Kumar et al., 2013). Another study showed a nonsignificant trend toward lower TNFα at 18 gestational weeks in future PE cases vs controls, but a significant increase at around 30 weeks of gestation (Serin et al., 2002). It is unknown whether the statistical increase we observed has a biological impact on GH. Our results need to be corroborated by other studies characterizing TNFα more frequently over the course of the pregnancy, while strictly defining GH and PE status, as we did.

Neither V1 nor V2 TNFα was different among the three groups, in accordance with some studies (Serin et al., 2002; Freeman et al., 2004; Ozler et al., 2012), but in contrast to others (Omu et al., 1999; Hamai et al., 1997). This may be explained by the fact that most studies that found higher TNFα in PE mothers measured TNFα at the third trimester (28–40 gestational weeks) (Xie et al., 2011) suggesting that higher maternal TNFα is a consequence of PE. The timing of sampling could explain the absence of difference observed here, as we measured TNFα in the first and second trimesters.

Our study has many strengths, including its prospective design, refined phenotyping, standardized measurement of blood pressure, and each case of GH or PE was reviewed in detail with a nephrologist. In addition, we are confident that our cohort is representative of other European-descent populations, as the incidence rate of HDP is within the expected range and our hypertensive participants presented predictable risk factors, namely primiparity, higher first-trimester BP, and excess weight. A limit of our study is that we solely measured TNFα as a marker of inflammation—other markers could also have been informative; however, we expect that TNFα is a good reflection of other known upstream inflammatory agents, such as toll-like receptors and thromboxane A_2.

In conclusion, we demonstrated that newborns from mothers diagnosed with PE have higher levels of TNFα at birth, independent of gestational age at delivery and maternal BMI. Cord blood TNFα at birth, as a biomarker of gestational systemic inflammation, could reflect the impact of PE on the fetus.

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