Determining Whether Hypertensive Status and Stress Level Are Associated With Inflammatory Markers

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Abstract

Background: Hypertensive disorders are common pregnancy complications in the United States. Although the exact mechanism underlying hypertensive disorders in pregnancy is unknown, there is evidence of involvement of a maladaptive maternal inflammatory response. Psychological maternal stress experienced during pregnancy can increase the risk of a hypertensive disorder by altering the maternal inflammatory response. **Objectives:** The purpose of this analysis was to evaluate the relationships of hypertensive status and stress with inflammatory biomarkers throughout pregnancy. **Method:** A 1:2 case-control design was used to analyze secondary data longitudinally with repeated measures of a multicenter, culturally and ethnically diverse pregnant population. Demographic data, psychological stress, and serum inflammatory data were analyzed. The sample consisted of 30 pregnant women with hypertension and 61 normotensive women. Measurements were taken once in each trimester of pregnancy. **Results:** Trimester-specific levels of inflammatory biomarkers varied based on stress and hypertensive status. IL-6 was elevated in the hypertensive, high-stress group, while IL-8 was greater among those with high stress, regardless of hypertensive status or trimester. For IL-1 α and IL-1 β , there was a significant stress-by-trimester interaction, while IL-10 was associated with a significant three-way interaction among stress level, hypertension status, and trimester. **Conclusions:** The associations of stress and hypertensive status with inflammatory biomarkers are complex. Stress and hypertension were associated with changes in inflammatory response. Hypertensive women with high stress experienced a heightened anti-inflammatory response, potentially a compensatory mechanism. To better understand this relationship, further longitudinal studies are warranted.

Keywords

inflammatory biomarkers, stress, pregnancy, hypertension

Hypertensive disorders are common complications in pregnancy, occurring in 5–10% of all pregnancies in the United States (Kuklina, Ayala, & Callaghan, 2009). These high-risk complications are associated with increased maternal and fetal morbidity and mortality and are a risk factor for the development of maternal cardiovascular disease later in life (Eiland, Nzerue, & Faulkner, 2012). In the United States, the rate of pregnancy-related mortality from hypertensive disorders in 2012 was 7.6% (Centers for Disease Control and Prevention, 2016). Hypertension during pregnancy is categorized as chronic hypertension, gestational hypertension, preeclampsia (including mild, superimposed, and severe forms), and eclampsia. Preeclampsia is the most prevalent hypertensive disease of pregnancy (Pennington, Schlitt, Jackson, Schulz, & Schust, 2012), with 17% of patients with gestational hypertension developing preeclampsia (Lo, Mission, & Caughey, 2013).

Although the exact mechanisms underlying preeclampsia remain unclear, there is evidence of the involvement of a maladaptive maternal inflammatory response. Systemic

inflammation is common to all pregnancies, but authors have proposed that preeclampsia results from an imbalance in this response (Redman, Sacks, & Sargent, 1999). Researchers have demonstrated an association between inflammation and the endothelial dysfunction linked with preeclampsia, more specifically an increased pro-inflammatory response.

One of the factors that may adversely influence the inflammatory response is maternal perceived stress. Psychological stress may increase the risk of preeclampsia by increasing

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activation of the maternal inflammatory system (Christian, 2012; Coussons-Read, Okun, & Simms, 2003; Crosson, 2012; Zhang et al., 2013). Limited data exist measuring these phenomena at each trimester during pregnancy. Substantial changes occur in maternal immune function over the course of a pregnancy; for this reason, understanding the inflammatory process in each trimester would be beneficial (Christian, 2012). Since hypertension and stress may alter the inflammatory response during pregnancy, leading to poorer outcomes, a better understanding of the inflammatory response may lead to early detection of complications. Therefore, the purpose of the present study was to determine the associations of hypertensive status and stress level with inflammatory biomarkers throughout the course of pregnancy.

Background

The severity of preeclampsia varies depending upon various factors such as gestational age at time of disease onset and the existence of comorbidities that affect pregnancy outcomes. Women who develop the disease after 36 weeks' gestation are less likely to experience adverse outcomes than women who develop the disorder before 33 weeks' gestation (Sibai, Dekker, & Kupferminc, 2005). Additionally, researchers found that the rate of fetal death was approximately 6 times higher (adjusted odds ratio 5.8; 95% confidence interval [CI] = [4.0, 8.3]) among women with early onset of the disease (Lisonkova & Joseph, 2013).

Preeclampsia can also have long-term maternal effects. A history of preeclampsia is associated with double the maternal risk of developing cardiac, cerebrovascular, and peripheral vascular disease compared to women without this risk factor (Eiland et al., 2012; Giguère et al., 2012; Gilbert et al., 2008). In addition, women with a history of preeclampsia have a 3- to 5-fold increased risk of developing metabolic syndrome later in life (Giguère et al., 2012).

Normal pregnancy results in changes in maternal physiology, including changes in immune response. A shift toward an anti-inflammatory response and inhibition of a pro-inflammatory response is associated with normal pregnancy (Southcombe, Redman, Sargent, & Granne, 2015). Researchers have hypothesized that preeclampsia is caused by a maladaptive maternal immune response to pregnancy, specifically exaggerated inflammation (Eiland et al., 2012; Palm, Axelsson, Wernroth, Larsson, & Basu, 2013). The condition is associated with an overall pro-inflammatory systemic environment with elevated levels of pro-inflammatory cytokines (Szarka, Rigó, Lázár, Bekő, & Molvarec, 2010). Researchers found that levels of tumor necrosis factor α (TNF α) increased in the second and third trimesters in women with preeclampsia (Moreli, Ruocco, Vernini, Rudge, & Calderon, 2012; Palm et al., 2013; Szarka et al., 2010). Likewise, levels of cytokines interleukin-6 (IL)-6 and IL-8 increased, while those of IL-10 decreased in the third trimester or at preeclampsia diagnosis (Borekci, Aksoy, Al, Demircan, & Kadanali, 2007; Kronborg et al., 2011; Szarka et al., 2010). These findings regarding serum biomarker levels

provide quantifiable measures of this pro-inflammatory response environment; however, few of these studies were longitudinal in design. Exploration of these changes throughout pregnancy, and prior to the development of preeclampsia, holds promise for early identification of and intervention for at-risk women.

Heightened psychological maternal stress experienced during pregnancy can increase the risk of preeclampsia. Physiological responses occur in the body with exposure to psychological stress (Crosson, 2012). These responses include the release of various inflammatory markers (Coussons-Read, Okun, & Nettles, 2007) and stress hormones such as cortisol (Crosson, 2012). Coussons-Read and colleagues (2003) hypothesized that maternal psychosocial stress during the prenatal period negatively affects pregnancy outcomes by altering the maternal immune response. Furthermore, the pooled effect of 12 studies indicated that maternal psychosocial stress was associated with an increased risk of preeclampsia ($OR = 1.49$; 95% CI [1.27, 1.74]; $p < .001$; Zhang et al., 2013). Elevated maternal psychosocial stress was also related to higher levels of IL-6 early and late in pregnancy and lower levels of IL-10 early in pregnancy (Coussons-Read et al., 2007). Unfortunately, the biomarkers measured are not consistent across studies, and the conclusions about their importance are thus equivocal.

There are limited data available regarding the associations between maternal stress and the inflammatory response measured longitudinally across all three trimesters and risk of the development of preeclampsia. Determining maternal stress levels in conjunction with measurement of trimester-specific immune markers may assist practitioners in identifying women at risk of the development of preeclampsia. Once a relationship between trimester-specific maternal stress and inflammatory response is established, early detection of preeclampsia may be possible, thereby increasing the likelihood of improved maternal outcomes.

Method

Design and Sample

The present study is a secondary analysis of a longitudinal, multicenter study of culturally and ethnically diverse pregnant women (Ashford et al., 2015). The primary aim of the parent study was to determine whether trimester-specific levels of inflammatory markers linked with psychosocial and biobehavioral variables can predict risk of preterm birth. The study was conducted at the University of Kentucky College of Nursing from 2010 to 2014 and was approved by the university's institutional review board.

For the present study, we used a 1:2 case-control design to analyze data collected in the parent study. We chose to use the 1:2 matching because controls far outnumbered cases in the full data set. Cases were women diagnosed with a hypertensive disorder of pregnancy, while controls were those with no such diagnosis. We frequency matched cases and controls on age (5-year increments) and parity (yes or no to prior pregnancy) in

the same trimester of pregnancy, randomly selecting controls from all normotensive participants who matched the age and parity profile of a given case. In all, we selected 33 women with a hypertensive disorder and 66 women without for this secondary analysis. Of these, we omitted eight from the analysis because they were missing all cytokine values, yielding a final sample size of 30 women with a hypertensive disorder (cases) and 61 without (controls).

Inclusion criteria for the parent study were pregnant women older than 16 years of age with a singleton gestation and currently on Medicaid (indicating low income). Exclusion criteria included history of Type 1 or Type 2 diabetes, history of any heart disease, current history of illegal or prescription drug abuse via urine drug screen, diagnosis of bacterial vaginosis or sexually transmitted disease, any autoimmune disease, and multifetal pregnancies. Women with chronic disease and/or multifetal pregnancy were excluded due to the significant association of these variables with preterm birth (Institute of Medicine, 2006); other exclusion criteria were related to alteration of the maternal immune response and/or were independent risk factors for preterm birth.

Procedures

Three prenatal clinics in Kentucky and Virginia served as recruitment sites for the parent study. Demographic data were collected at the first visit. Data collection periods comprised three prenatal office visits, which we retained for the present study: (1) 5–13 weeks', (2) 14–26 weeks', and (3) 27–36 weeks' gestation. There were at least 4 weeks between collection points. At each assessment, participants provided a serum sample and completed a stress questionnaire.

Serum samples were collected using standard venipuncture. For long-term storage, blood samples were centrifuged, pipetted into aliquots, and stored at -80°C . Samples were slowly thawed prior to analysis. Cytokine samples were analyzed undiluted. All samples were run in duplicate according to assay manufacturers' protocols (Ashford et al., 2015).

A stress questionnaire was used to assess maternal psychological stress. These data were collected immediately following biomarker collection at each trimester by trained staff. The staff gave the questionnaire to study participants to complete on their own during their office visit and provided verbal instructions. Participants could select a web-based survey or paper copy according to their preference. All written material was available in English and Spanish at a sixth-grade reading level.

Measures

Systemic inflammation. Serum inflammatory biomarkers, specifically the pro-inflammatory cytokines IL-1 α , IL-1 β , IL-6, IL-8, and TNF α and the anti-inflammatory cytokine IL-10, were measured using the Luminex system. The Luminex system is a highly reproducible and reliable bead-based assay that enables quantification of multiple proteins simultaneously

(Tighe, Negm, Todd, & Fairclough, 2013). When compared to the gold standard of cytokine measurement, enzyme-linked immunosorbent assay, the multiplex bead array has a high correlation coefficient, ranging from 0.912 to 1.0 (Elshal & McCoy, 2006).

Maternal stress. Stress was defined as maternal psychological stress during pregnancy and was measured using the everyday stressors index (ESI; Hall, 1983). The ESI is a reliable and valid 20-item self-report questionnaire used to evaluate stress during the perinatal period in low-income mothers (Hall, 1983; Peden, Rayens, Hall, & Grant, 2004; Pollock, Amankwaa, & Amankwaa, 2005). The index assesses five common sources of maternal stress including role overload, financial concerns, parenting worries, employment problems, and interpersonal conflicts. Respondents rate how much each of 20 stressors worried, upset, or bothered them on a day-to-day basis on a 4-point response scale ranging from *not bothered at all* (0) to *bothered a great deal* (3). A cumulative score is obtained by summing the scores for all of the items, for a possible range of 0–60, with higher scores reflecting higher levels of stress.

Demographic characteristics. The demographic factors of age, race/ethnicity (White/non-Hispanic vs. Other), education (high school or less vs. postsecondary), income level in US ($\geq \$30,000$ vs. $< \$30,000$), and marital status (married/partnered vs. not) were collected via self-report at the first-trimester appointment.

Data Analysis

Descriptive analysis included means and standard deviations or frequency distributions. Given the skewed nature of inflammatory-marker measures and maternal stress, we investigated transformations. We successfully transformed serum inflammatory-marker values by taking the natural log prior to analysis and descriptively summarized these variables using geometric means and geometric standard deviations. For maternal stress, we averaged scores over the three assessments to evaluate overall degree of stress during the prenatal period. Because the maternal stress score (ESI) remained skewed even after several different transformation strategies, we categorized this average score into high stress (at or above 75th percentile) and low stress (below 75th percentile) using a cut point of 3.

To compare demographic characteristics between women with a hypertensive disorder (cases) and those without (controls), we used two-sample *t* tests and χ^2 tests of association. To discern differences in inflammatory biomarker levels by stress level and hypertension status over the trimesters throughout pregnancy, we used repeated-measures mixed modeling via the MIXED procedure in SAS. The between-subject factors for each mixed model included stress level (high/low) and having a hypertensive disorder (yes/no) during pregnancy. For each cytokine, the initial model contained these main effects and the main within-subject effect of time (trimester) as well as their two- and three-way interactions. For all models in which the

Table 1. Sample Characteristics and Group Comparisons of the Subgroups Formed by Hypertensive Status During Pregnancy.

Characteristic	Hypertensive Disorder (<i>n</i> = 30)	Normotensive (<i>n</i> = 61)	<i>p</i>
Age, years (mean $\pm$ SD)	26.3 $\pm$ 4.3	26.2 $\pm$ 4.1	.86 ^a
Race, <i>n</i> (%)			.83 ^b
White/non-Hispanic	23 (77)	48 (79)	
Other	7 (23)	13 (21)	
Education, <i>n</i> (%)			.97 ^b
$\leq$ High school	6 (20)	12 (20)	
>High school	24 (80)	49 (80)	
Income, <i>n</i> (%)			.95 ^b
<\$30,000	12 (40)	24 (41)	
$\geq$ \$30,000	18 (60)	35 (59)	
Marital status, <i>n</i> (%)			.053 ^b
Single/not living with partner	3 (10)	17 (28)	
Married/living with partner	27 (90)	44 (72)	
ESI composite score, first trimester (mean $\pm$ SD)	5.1 $\pm$ 7.7	2.6 $\pm$ 4.8	.11 ^a

Note. *N* = 91. ESI = everyday stressors index.

^aComparison via two-sample *t* test. ^bComparison via χ^2 test of association.

three-way interaction was not significant, we evaluated a final model with this term omitted. We conducted post hoc pairwise comparisons for significant main or interaction effects using Fisher's least significant difference procedure. We used SAS Version 9.4 for all analyses, using an α of .05 throughout.

Results

Table 1 shows the sociodemographic characteristics and baseline stress scores for each hypertensive-status group. Per the 2:1 matching, this sample consisted of 30 (33%) pregnant women with a hypertensive disorder and 61 (67%) without. The average age across the groups was 26.2 years (*SD* = 4.2). Most participants were White/non-Hispanic (78%) with at least some postsecondary education (80%). The majority had annual incomes greater than \$30,000 (60%) and were married or living with a partner (78%). The average baseline stress score for all participants was 3.4 (*SD* = 6.0). While women in the hypertensive group were more likely to be married/partnered and have a higher ESI composite score, none of the group comparisons shown in Table 1 are statistically significant. The groups were thus comparable in terms of demographic characteristics and baseline stress.

Serum Cytokine Levels by Hypertensive Status and Stress Level Over Pregnancy

Table 2 provides details from the repeated-measures models. For both IL-1 α and IL-1 β , the three-way interaction in the repeated-measures mixed model was not significant, and the only significant two-way or main effect in the final model was the stress-by-trimester interaction. For IL-1 α , the post hoc analysis suggests that, among those in the high-stress group, the

first-trimester IL-1 α level was significantly elevated compared to the levels in both the second (p = .007) and third trimesters (p = .009), but the levels in the latter two trimesters did not differ from each other. For this cytokine, there were no significant pairwise differences among the trimesters for those in the low-stress group, but the high- and low-stress groups differed significantly in the first trimester (with the high-stress group having a larger average value than the low-stress group, p = .006) but not in the subsequent trimesters. For IL-1 β , the only significant pairwise differences in the post hoc analysis were within the low-stress group, with the average value for this cytokine during the third trimester being significantly greater than at each of the earlier trimesters (p = .02 for the comparison to the first-trimester levels, and p = .04 for the comparison to second-trimester levels). No other relevant comparisons, including within the high-stress group and between the two stress groups for a fixed trimester, were significant.

For the cytokine IL-6, the two-way interaction between stress and hypertensive status was significant. The post hoc analysis indicated that, among those with high stress, the subgroup who also had hypertension had an elevated mean IL-6 level compared to those without hypertension (p = .02). An additional significant comparison suggested that, among those with hypertension, the subgroup of participants who were also in the high-stress group had greater IL-6 levels compared to those with low stress (p = .02). Together, these two results suggest that participants with both hypertension and high stress have higher IL-6 values than those who had just one of these. The only significant effect in the IL-8 model was the main effect of stress level. Those in the high-stress group had elevated IL-8 levels compared to those with low stress, regardless of hypertensive status or trimester.

The three-way interaction among stress, hypertensive status, and trimester was significant in the IL-10 model. The relevant pairwise comparisons indicated that, among those without hypertension, during both the first and second trimesters, the low-stress group had higher IL-10 levels than the high-stress group (p = .03 and .008 for the first- and second-trimester comparisons, respectively). In this same post hoc analysis, among those with high stress and hypertension, the first- and second-trimester mean IL-10 levels were greater than the mean level in the third trimester (p = .05 and .01 for first and second trimesters, respectively). No other relevant pairwise comparisons were significant in this model.

Finally, none of the main or interaction effects were significant for the TNF- α model, so post hoc analysis was not warranted.

Descriptive Summary of Cytokine Values

Tables 3 through 5 provide descriptive summaries of the geometric means and geometric standard deviations for cytokine scores among various subgroups of participants. As shown in Table 3, for the high-stress and low-stress groups combined, there is a tendency for cytokine values to be elevated in the hypertensive group (relative to the normotensive participants),

Table 2. Repeated-Measures Models for Each Serum Inflammatory Biomarker With Main Effects of Hypertension (Yes/No), Stress (High/Low), and Trimester.

Biomarker	Hypertensive Status		Stress Level		Trimester		Stress × Trimester		Stress × Hypertensive Status		Stress × Hypertensive Status × Trimester	
	F	p	F	p	F	p	F	p	F	p	F	p
IL-1 α	<0.1	.93	2.2	.14	2.5	.09	5.0	.008*	—	—	—	—
IL-1 β	0.1	.78	<0.1	.92	1.1	.32	3.2	.04*	—	—	—	—
IL-6	3.4	.07	2.7	.10	0.4	.70	—	—	4.4	.04*	—	—
IL-8	<0.1	.88	7.6	.007*	0.7	.51	—	—	—	—	—	—
IL-10	0.1	.78	3.7	.06	0.2	.86	—	—	—	—	3.3	.04*
TNF- α	0.3	.62	0.8	.37	2.0	.14	—	—	—	—	—	—

Note. Two-way interactions included in all models but not shown here unless $p < .05$. IL = interleukin; TNF- α = tumor necrosis factor α .

* $p < .05$.

Table 3. Geometric Mean and Geometric Standard Deviation for Serum Inflammatory Biomarkers at Each Trimester by Hypertensive Status.

Cytokine	Trimester 1: Mean $\pm$ SD (pg/ml)		Trimester 2: Mean $\pm$ SD (pg/ml)		Trimester 3: Mean $\pm$ SD (pg/ml)	
	Hypertensive (n = 30)	Normotensive (n = 61)	Hypertensive (n = 28)	Normotensive (n = 56)	Hypertensive (n = 25)	Normotensive (n = 51)
IL-1 α	2.65 $\pm$ 10.78	1.89 $\pm$ 8.08	1.73 $\pm$ 7.12	1.72 $\pm$ 8.18	2.14 $\pm$ 7.61	1.94 $\pm$ 8.64
IL-1 β	1.10 $\pm$ 5.24	0.79 $\pm$ 6.27	0.92 $\pm$ 4.98	0.74 $\pm$ 6.60	0.76 $\pm$ 5.38	1.49 $\pm$ 5.60
IL-6	6.49 $\pm$ 9.30	2.80 $\pm$ 4.69	4.65 $\pm$ 7.46	2.92 $\pm$ 6.91	4.34 $\pm$ 7.93	3.35 $\pm$ 7.83
IL-8	9.74 $\pm$ 3.27	6.94 $\pm$ 3.05	6.61 $\pm$ 2.99	7.34 $\pm$ 3.09	7.32 $\pm$ 3.13	10.01 $\pm$ 3.08
IL-10	7.40 $\pm$ 6.47	6.59 $\pm$ 4.92	7.17 $\pm$ 6.19	7.18 $\pm$ 5.21	5.04 $\pm$ 9.17	7.90 $\pm$ 4.54
TNF- α	6.58 $\pm$ 2.95	6.96 $\pm$ 2.95	7.85 $\pm$ 2.53	7.56 $\pm$ 3.29	9.70 $\pm$ 1.94	9.74 $\pm$ 2.52

Note. IL = interleukin; TNF- α = tumor necrosis factor α .

Table 4. Geometric Mean and Geometric Standard Deviation for Serum Inflammatory Biomarkers Among Normotensive Women at Each Trimester by Stress Group.

Cytokine	Trimester 1: Mean $\pm$ SD (pg/ml)		Trimester 2: Mean $\pm$ SD (pg/ml)		Trimester 3: Mean $\pm$ SD (pg/ml)	
	Low Stress (n = 49)	High Stress (n = 12)	Low Stress (n = 45)	High Stress (n = 11)	Low Stress (n = 40)	High Stress (n = 11)
IL-1 α	1.32 $\pm$ 5.51	8.02 $\pm$ 17.65	1.54 $\pm$ 7.12	2.64 $\pm$ 14.36	1.96 $\pm$ 7.81	1.86 $\pm$ 13.46
IL-1 β	0.67 $\pm$ 5.88	1.60 $\pm$ 7.43	0.72 $\pm$ 6.98	0.83 $\pm$ 5.60	1.71 $\pm$ 5.31	0.91 $\pm$ 6.69
IL-6	2.74 $\pm$ 3.77	3.08 $\pm$ 10.06	3.00 $\pm$ 5.98	2.60 $\pm$ 12.66	3.64 $\pm$ 7.11	2.50 $\pm$ 11.70
IL-8	6.02 $\pm$ 2.91	12.38 $\pm$ 3.21	6.72 $\pm$ 3.20	10.52 $\pm$ 2.56	9.36 $\pm$ 3.17	12.77 $\pm$ 2.78
IL-10	8.29 $\pm$ 3.29	2.58 $\pm$ 12.80	9.25 $\pm$ 3.78	2.55 $\pm$ 10.99	10.26 $\pm$ 3.63	3.06 $\pm$ 6.81
TNF- α	7.48 $\pm$ 2.92	5.18 $\pm$ 3.06	8.27 $\pm$ 3.29	5.22 $\pm$ 3.25	11.23 $\pm$ 2.22	5.79 $\pm$ 3.26

Note. IL = interleukin; TNF- α = tumor necrosis factor α .

particularly during the first and second trimesters. For some cytokines, this relationship is reversed by the third trimester.

Among normotensive participants, those with high stress tended to have elevated cytokines compared with those who scored low on the stress measure (see Table 4). This observation was generally true for IL-1 α , IL-1 β , IL-6, and IL-8, except for a reversal between the groups in the third trimester for IL-1 α , IL-1 β , and IL-6. The pattern was different for IL-10 and TNF- α , which were higher for the low-stress group in general, regardless of trimester. The pattern among hypertensive women was generally the same, with higher geometric means in the high relative to the low-stress group (see Table 5). For

this subgroup of women with a hypertensive disorder, only TNF- α exhibited the flip from lower to higher scores between the first and third trimester, and this was only within the low-stress group.

Discussion

This study is the first to date that has examined the combined effects of stress, hypertension status and time (trimester) on inflammatory biomarkers. The aim of this secondary analysis was to examine the relationship of inflammation with other conditions that can present during pregnancy. We used

Table 5. Geometric Mean and Geometric Standard Deviation for Serum Inflammatory Biomarkers Among Hypertensive Women at each Trimester by Stress Group.

Cytokine	Trimester 1: Mean $\pm$ SD (pg/ml)		Trimester 2: Mean $\pm$ SD (pg/ml)		Trimester 3: Mean $\pm$ SD (pg/ml)	
	Low Stress (<i>n</i> = 20)	High Stress (<i>n</i> = 10)	Low Stress (<i>n</i> = 17)	High Stress (<i>n</i> = 11)	Low Stress (<i>n</i> = 15)	High Stress (<i>n</i> = 10)
IL-1 α	1.88 $\pm$ 9.12	5.27 $\pm$ 14.40	1.38 $\pm$ 4.83	2.45 $\pm$ 12.13	1.62 $\pm$ 6.90	3.24 $\pm$ 9.06
IL-1 β	1.03 $\pm$ 5.13	1.26 $\pm$ 5.92	0.94 $\pm$ 5.65	0.88 $\pm$ 4.34	0.76 $\pm$ 6.62	0.76 $\pm$ 4.10
IL-6	3.22 $\pm$ 6.94	26.44 $\pm$ 8.99	2.16 $\pm$ 4.10	15.25 $\pm$ 9.69	2.78 $\pm$ 5.98	8.48 $\pm$ 10.73
IL-8	7.76 $\pm$ 3.22	15.36 $\pm$ 3.14	4.02 $\pm$ 2.16	14.24 $\pm$ 3.04	5.43 $\pm$ 3.48	11.45 $\pm$ 2.27
IL-10	7.40 $\pm$ 4.57	7.39 $\pm$ 12.43	7.24 $\pm$ 4.34	7.08 $\pm$ 10.50	6.37 $\pm$ 10.16	3.54 $\pm$ 8.36
TNF- α	5.81 $\pm$ 3.05	8.45 $\pm$ 2.78	6.89 $\pm$ 2.74	9.61 $\pm$ 2.22	12.19 $\pm$ 1.66	6.88 $\pm$ 2.12

Note. IL = interleukin; TNF- α = tumor necrosis factor α .

repeated-measures models to evaluate these relationships and also descriptive analysis to summarize cytokine values over time within subgroups of participants.

Our findings indicate that, among the high-stress group, first-trimester IL-1 α level was elevated compared to the levels in the second and third trimesters. Regardless of hypertension status, there was a significant interaction between stress level (high/low) and trimester for this cytokine. This finding partially supports Coussons-Read and colleagues' (2003) hypothesis that maternal perceived stress during the prenatal period has a negative effect on pregnancy outcomes through alteration of the maternal immune response. Stress level, alone, also had an effect on this pro-inflammatory biomarker, as its levels were elevated in the high compared to the low-stress group in the first trimester. There was no difference over time in IL-1 α levels among those in the low-stress group.

Women with low-stress scores had significantly higher levels of IL-1 β in the third trimester compared to earlier trimesters, but in the high-stress group, differences across trimesters for IL-1 β were not significant. Coussons-Read, Okun, and Nettles (2007) previously published results in which IL-1 β level was higher in pregnant women experiencing higher levels of stress. It is important to note the instruments used to measure stress differed between the two studies and may not have captured the same dimensions. In addition, the women in the present study—even those in the high-stress group—had relatively low-stress scores, while the women in the Coussons-Read study were equally distributed across low-, average-, and high-stress groups.

Women with a hypertensive disorder and high levels of stress had higher levels of IL-6 across all trimesters compared to women with either high stress or a hypertensive disorder. Coussons-Read and colleagues' (2007) found that pregnant women experiencing higher levels of stress had higher IL-6 levels than those with lower levels of stress, but they did not include the presence or absence of hypertensive disorders in their analysis. Other researchers have reported increased levels of IL-6 during the last trimester in hypertensive women without the effect of stress (Szarka et al., 2010). IL-8 was elevated in the high-stress group relative to the low-stress group, regardless of hypertensive status or trimester in the present study. To

date, no other studies have measured IL-8 in the context of high stress in a hypertensive pregnancy.

There was a significant three-way interaction among stress level, hypertension status, and trimester for IL-10 only. Serum levels of IL-10 were significantly higher in the third trimester in women with high stress and a hypertensive disorder compared to normotensive women with low-stress levels. We would expect IL-10 production to be heightened in the third trimester until just prior to onset of labor in normal pregnancy (Moreli et al., 2012). However, this finding of an exaggerated anti-inflammatory response in the third trimester in hypertensive women with high stress is an indication of dysregulation of the inflammatory response and might be a compensatory mechanism (Szarka et al., 2010).

Research has previously shown that high levels of stress are associated with a high-risk pregnancy, such as one complicated with a hypertensive disorder (Cardwell, 2013). However, in the *t*-test comparison in the present study of those with and without a hypertensive disorder, the level of maternal stress did not significantly differ between the groups. We provide the geometric means and standard deviations for the serum inflammatory biomarker levels at each trimester by hypertensive status (Table 3) along with separate data for the stress subgroups within each hypertensive-status group (Tables 4 and 5). These data may inform future work as they provide a map of the cytokines that are most and least variable across trimesters within each of these subgroups.

Limitations

As it was a secondary data analysis, some aspects of this study were not optimal. A diagnosis of a hypertensive disorder was not an integral component of the parent study. Therefore, the sample size of women with a hypertensive disorder was small, especially when collecting longitudinal measures, given the loss of some participants at each successive time point. We included participants with preeclampsia in the hypertensive-disorder group in order to bolster the sample size; however, even with this convention, the number of women in the hypertensive group was relatively small. In addition, we dichotomized the stress measure to high/low stress prior to analysis

due to its skewness. Future studies might benefit from using a more sensitive assessment of maternal stress. The retrospective nature of the design did not allow for power estimation (Hoenig & Heisey, 2001); additional work in this area would be strengthened by using a prospective design and larger sample size. Consistent with other studies, although inflammatory biomarker data were evaluated for each trimester, there was variability among participants in the serum measures for a given trimester. This could be due to the 6-week collection window within each trimester, as these concentrations may vary by gestational age. Finally, its possible serum levels of these inflammatory biomarkers do not accurately capture differences between women developing a hypertensive disorder and those who are not, while other mediums, such as cervicovaginal fluid, may be more representative of the physiological maternal response. Future studies will benefit from including more than one medium to test for consistency of results.

Conclusions

Systemic inflammation occurs during pregnancy; however, an imbalance in the inflammatory response might lead to adverse maternal outcomes. Our findings in the present study provide preliminary evidence that both stress and hypertension play a role in the regulation of this inflammatory process during pregnancy. For example, hypertensive women with high stress experienced a heightened anti-inflammatory response, as indicated by IL-10 levels, compared to normotensive women and those with low-stress levels, potentially indicating a compensatory mechanism.

The effects of an association between subjective stress and levels of inflammatory biomarkers on the development of a hypertensive disorder are complex. Although the exact cause of these disorders is unclear, research has demonstrated associations between a maladaptation of the maternal immune response, specifically an exaggerated pro-inflammatory response, and both the development of a hypertensive disorder and a heightened stress response. To understand this relationship more thoroughly, further longitudinal studies with larger sample sizes are warranted. A better understanding of this relationship might aid in early detection and intervention for women at risk of developing this pregnancy complication.

Author Contributions

S. Kehler contributed to concept, design, analysis, and interpretation of data; drafted the manuscript; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of the work in ensuring that questions relating to the accuracy or integrity of any part of the work are appropriately investigated and resolved. M. K. Rayens contributed to analysis and interpretation, critically revised the manuscript, gave final approval, and agreed to be accountable for all aspects of work ensuring integrity and accuracy. K. Ashford contributed to conception, design acquisition, analysis, and interpretation; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

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