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Mid-pregnancy Circulating Immune Biomarkers in Women with Preeclampsia and Normotensive Controls

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Abstract

Objectives—To determine if mid-pregnancy circulating immune biomarkers are associated with preeclampsia.

Study Design—Nested case-control study of 410 preeclamptic women and 297 normotensive controls with primiparous singleton pregnancies enrolled in the Danish National Birth Cohort. The mean gestational age in our cohort is 16 weeks (range 9–26).

Main Outcome Measures—Preeclampsia was defined by blood pressure ≥ 140/90mmHg and proteinuria ≥3g/24h. Serum immune biomarkers included interleukin (IL)-6, IL-6 receptor, IL-4, IL-4 receptor, IL-5, IL-12, IL-2, TNF-α, TNF-β, TNF-receptor, IL-1β, IL-1α, IL-8, IL-10, IFN-γ, IL-18, macrophage migration inhibitory factor, macrophage inflammatory protein, transforming growth factor-beta (TGF-β), and RANTES. Associations with preeclampsia, term preeclampsia

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and preterm preeclampsia were determined using two logistic regression models; 1) biomarkers were dichotomized by the limit of detection (LOD): 2) on the continuous scale, non-detectable values were imputed by LOD/2 and transformed (base 2). All models were adjusted for body mass index and smoking.

Results—IL1β was significantly associated with a decrease in the log odds of preeclampsia (p=0.0065), term preeclampsia (p=0.0230) and preterm preeclampsia (p=0.0068). Results were similar for IL4r and preeclampsia (p=0.0383). In the dichotomized models, detectable TNF-β was significantly associated with preeclampsia (OR_{adj} 1.6, 95% CI 1.1–2.3) and term preeclampsia (OR_{adj} 1.7, 95% CI 1.1–2.5) but not preterm preeclampsia. Detectable IL6 was significantly associated with term preeclampsia only (OR_{adj} 1.5, 95% CI 1.1–2.2).

Conclusion—Mid-pregnancy circulating IL1β, IL4r, IL6, and TNFβ were associated with preeclampsia. However, results were not consistent across statistical models. As the relationship is complex, future studies should explore cytokine clusters in preeclampsia risk.

Keywords

Cytokines; Inflammation; Preeclampsia; Pregnancy

INTRODUCTION

Preeclampsia is a maternal syndrome clinically characterized by the new onset of hypertension and proteinuria after 20 weeks of gestation. It affects approximately 3–5% of pregnancies and is a leading cause of fetal and maternal morbidity and mortality [1]. Delivery of the fetus, which is often preterm, is the only treatment and there are no biomarkers which can adequately predict risk. Thus, preeclampsia remains a serious public health burden.

The etiology of preeclampsia is unknown but several subtypes likely exist and may be classified, although broadly, as maternal, placental, or both [2]. Preeclampsia is suggested to be a state of exaggerated systemic inflammation [3] and possibility a condition that is biased towards Th1/Th17 predominance [4]. An inappropriate immunological interaction early in pregnancy is one pathway hypothesized to lead to abnormal placentation and the production of materials released into the maternal circulation causing systemic inflammation, endothelial dysfunction and maternal disease [2]. Maternal factors such as infection or obesity may also contribute to systemic inflammation in preeclamptic women which could result in oxidative stress and endothelial dysfunction [5, 6].

As the immune system is complex, studies have focused on concurrently examining several circulating immune biomarkers with inflammatory, anti-inflammatory or immune-modulatory functions in preeclampsia, with particular focus on cytokines. Cytokines, soluble proteins with multiple immunological functions, are suggested to be involved in embryo implantation, trophoblast growth and differentiation and parturition [7]. In preeclampsia, an increase in circulating pro-inflammatory cytokines could activate endothelium triggering an exaggerated systemic inflammatory response [3]. This is consistent with third trimester studies reporting increased levels of circulating pro-inflammatory biomarkers such as tumor

necrosis factor alpha (TNFα), interleukin (IL)-8, IL-1β, macrophage inhibitory protein (MIP), and interferon (IFN)-γ in preeclampsia cases [8–10]. However, circulating levels of cytokines with anti-inflammatory functions such as IL-10 and IL1ra have been reported in similar studies [8, 11]. In addition, several pleiotropic and immune-modulatory biomarkers including IL-6, IL-2, IL-4, and IL-18 have been implicated in preeclampsia [8–10].

Although small case-control studies have reported significant associations between various immune biomarkers and preeclampsia in the third trimester,[8, 11–17] these results have not been replicated in other investigations [18–23]. The most consistent associations have been found with IL-6, TNFα and IL-8. However, investigations conducted prior to preeclampsia diagnosis have not found consistent results [24, 25]. One longitudinal study of 99 women began sampling at 18 weeks and found inconsistent increases in TNF-α and IL-6 across pregnancy in women who developed preeclampsia [26]. In part, these contrasting findings may be due to the measurement of cytokines from various biological samples, differences in assay methods and sample size. Our objective was to examine associations between 20 mid-pregnancy inflammatory, anti-inflammatory or immune-modulatory biomarkers and preeclampsia in a large sample of women who participated in the Danish National Birth Cohort.

MATERIAL AND METHODS

This study was part of a previously completed nested case control study of 562 women with preeclampsia and 377 normotensive controls from the Danish National Birth Cohort (DNBC) [27]. In this study, both cases and controls were primiparous with singleton pregnancies, had no gestational hypertension and no history of hypertension. The DNBC is a longitudinal population-based cohort of pregnant women and their offspring who were recruited at first prenatal visits (median 16 weeks, range 6–40 weeks) between 1996 and 2003 [28]. Gestational age was determined by the last menstrual period but corrected with an early ultrasound if neeeded. The DNBC was approved by the Danish Ethics Central Committee.

For our analysis, we were able to obtain data on immune biomarkers from 410 women with preeclampsia and 297 normotensive controls. The majority of samples in our study were collected in the second trimester (n=636; median 17 weeks) and an additional 71 samples were from women recruited in the first trimester (median 12 weeks). We conducted our analysis by pooling these samples together, since inclusion of the relatively small number of first trimester samples was not expected to significantly influence the results and the mean gestational age was 16.7 weeks (range10–26) in controls and 16.8 weeks (range 9–26) in cases. This study was approved by the University of Pittsburgh Institutional Review Board and the Danish Data Protection Agency.

In the DNBC, preeclampsia was determined by a positive report of preeclampsia at the postnatal interview (blood pressure ≥140/90mmHg measured twice with an interval of at least 6 hours and the presence of proteinuria (≥0.3 g/24 hours or 1+ urine dipstick measured twice with an interval of at least 4 hours). Reported preeclampsia is then confirmed by an *International Classification of Diseases* (ICD) discharge diagnoses in the National Hospital

Discharge Registry of 637.03, 637.04, 637.09, 637.19 (ICD-8) or D014 to D015 (ICD-10). This method has been shown to be highly specific for the diagnosis of preeclampsia (99%) when compared to the American College of Obstetrics and Gynecology (ACOG) criteria [29]. As a separate outcome, we considered preterm preeclampsia (preeclampsia resulting in a preterm birth < 37 weeks of gestation) as a proxy for time of onset of disease.

Whole blood samples were obtained at the first study visit and then mailed to the Statens Serum Institute in Copenhagen. Samples were stored at −80°C with an average time of < 28 hours from collection to processing. Serum immune biomarkers including IL-6, IL-6 receptor, IL-4, IL-4 receptor, IL-5, IL-12, IL-2, TNF-α, TNF-β, TNF-receptor, IL-1β, IL-1α, IL-8, IL-10, IFN-γ, IL-18, macrophage migration inhibitory factor (MIF), macrophage inflammatory protein (MIP), transforming growth factor-beta (TGF-β), and RANTES were included in our study based on previous associations with preeclampsia or their involvement in systemic inflammation or the Th1/Th17 paradigm [3, 4, 25]. All biomarkers were measured in duplicate with an in house multiplex flow cytometric assay system Luminex MultiAnalyte Profiling Technology (LabMap, Luminex Corporation, Austin Texas) [30]. The calibration curves for each analyte were calculated by the Bio-Plex 3.0 software (BioRad, US). Mean intra- and interassay CVs (CV %) were 6.2% and 16%, and ranged from 6.7 (IL-4) to 13 (IL-10 and TNF-α) and 10 (IL-4) to 25 (TNF-α) [30]. Variation in precision profiles among analytes is common and these results are similar to other studies [31–33]. In multiplex assays, subjects frequently measure outside the limit of detection (LOD) [34]. In general, the frequency of women who measure beyond the LOD varies and those who are outside the detectable range may have very low or undetectable levels. In our analysis, IL-2 was not considered in subsequent analysis because of scarce observations within its detectable range.

Telephone interviews were administered to participants in the DNBC at the first study visit, at 30 weeks gestation and then twice after delivery, when children were 6 and 18 months old. During the first study interview women reported their cigarette use during pregnancy and pre-pregnancy weight and height. Maternal age was self-reported at baseline and grouped as ≥25, 26–30, and 31+. Pre-pregnancy body mass index (BMI) was determined using reported height and weight at the first interview (median 16 weeks gestation) and was categorized as underweight or normal (<25), overweight (≥25 and <30), or obese (≥30).

Associations between baseline variables including maternal age, BMI, and smoking and preeclampsia were examined using a multivariable logistic regression model to derive odds ratios (OR) and 95% confidence intervals (CI) to identify potential co-variants. Logistic regression models with P-splines were used to examine association between gestational age at blood draw and odds of preeclampsia. As values beyond the LOD are common in the majority of the immune biomarkers under consideration, it is impossible to perform statistical analysis within a unified framework without various unverifiable assumptions about biomarker distributions. We examined our data utilizing two common approaches for values beyond the LOD [34]. First, we imputed non-detectable values by the LOD divided by 2 and transformed all biomarkers to the log base 2 (a unit increment represents doubling of intensity). Logistic regression was used to calculate effect size and p-values. We also dichotomized immune biomarkers by the LOD (with non-detects as the reference group) to

examined associations between detectable levels and preeclamptic using logistic regression. For multivariable models, we considered gestational age at blood draw, maternal age, BMI, smoking and small for gestational age as potential covariates. Only variables which changed the effect size by greater than 10% were included in our final models. Thus, our final models adjusted for BMI and smoking as inclusion of the other variables had no effect on our results. All analyses were repeated using multinomial logistic regression to examine associations with term and preterm preeclampsia with normotensive women as the reference group. All analyses were conducted using SAS V9.2 (Cary, NC).

RESULTS

Table 1 compares potential covariates between 409 preeclampsia cases and 297 normotensive controls. The mean gestational age of blood sampling was 16.8 weeks for controls and 16.9 weeks for cases. Women who reported smoking had a lower odds of preeclampsia (OR 0.6, 95% CI 0.3- 0.9). In addition, a BMI of 25–29 (OR 1.8, 95% CI 1.2–2.6) and a BMI of 30 or greater (OR 3.8, 95% CI 2.1 – 6.8) were both associated with higher odds of preeclampsia. Maternal age was not significantly associated with preeclampsia.

Table 2 provides descriptive statistics for each immune biomarker in normotensive and preeclamptic women as well as results from our continuous logistic regression model. Normotensive women have higher median levels of RANTES (38.9 vs. 36.1), IL4r (420.0 vs. 373.5), IL1β (270.0 vs. 214.5), and IL10 (252.0 vs. 225.5) but lower median levels of IFNγ (57.0 vs. 112.5), IL18 (2.5 vs. 2.9) TGFβ (821.0 vs. 1008.0) and TNFβ (499.0 vs. 514.5) compared to women with preeclampsia. All other immune biomarkers were similar between groups. After adjusting for BMI and smoking, IL4r [effect estimate −0.06 (p-value 0.0383) and IL1β [−0.12 (0.0068)] were significantly associated with a decrease in the log odds of preeclampsia. No other significant associations were observed.

Our dichotomous model was not entirely consistent (Table 3). Women with detectable levels of IL4r (OR_{adj} 0.7, 95% CI 0.4–1.0) and IL1β (OR_{adj} 0.6, 95% CI 0.3–1.0) had a decreased odds of preeclampsia. However, these associations were of borderline significance. Women with detectable levels of TNF-β (OR_{adj} 1.6, 95% CI 1.1–2.3) had increased odds of preeclampsia. Detectable levels of IL-6 (OR_{adj} 1.4, 95% CI 1.0–2.0) also increased the odds of preeclampsia but results were of borderline significance. No other significant associations were observed.

Among our 409 preeclamptic cases, 87 (21%) had a preterm delivery and 322 a term delivery. Normotensive women did have higher median levels of IL4r than women with preterm preeclampsia (353.0) and term preeclampsia (384.0) (Table 4). In contrast to our analysis of all preeclampsia cases, we found no significant associations between IL4r levels and preterm [−0.01 (0.6410) or term preeclampsia [−0.01 (0.0733)] after adjustments. We also found that median levels of TNFβ were higher (555.0 vs. 499.0) and significantly associated with term preeclampsia when compared to normotensive women [0.1 (0.0496)]. This was not observed for preterm preeclampsia [Median 370.0; −0.01(0.7571)]. Median IL1β levels were lower in both preterm (199.0) and term preeclampsia (229.0) compared to normotensive women (272.0). After adjustments, IL1β was significantly associated with a

decreased in the log odds of preterm [−0.17(0.0065)] and term preeclampsia [−0.10 (0.0230)]. We found no other significant associations.

In our dichotomized model, we found that detectable levels of TNFβ (OR_{adj} 1.7, 95% CI 1.1–2.5) and IL-6 (OR_{adj} 1.5, 95% CI 1.1–2.2), were significantly associated with an increased odds of term preeclampsia. We found borderline significant associations between detectable levels of TGFβ (OR_{adj} 1.4, 95% CI 1.0–2.0). In contrast, IL1β displayed a trend towards decreased odds of term preeclampsia (OR_{adj} 0.6, 95% CI 0.3–1.0). These associations were not observed for preterm preeclampsia, although there was a trend towards decreased odds for IL1β (OR_{adj} 0.5, 95% CI 0.2–1.2). No other significant associations were observed.

DISCUSSION

Our results show that with each unit increase in IL1β the log odds of preeclampsia, term preeclampsia and preterm preeclampsia significantly decreases. The dichotomized model revealed borderline significant results for IL1β. Results were similar for IL4r but only in all preeclampsia cases. We found associations between detectable levels of TNFβ with both preeclampsia and term preeclampsia but not preterm preeclampsia. Detectable IL6 was associated with term preeclampsia only. These results were not consistent with our continuous model. Overall, none of these immune biomarkers are likely to have a strong predictive value.

IL-1β was associated with decreased odds of preeclampsia, term preeclampsia and preterm preeclampsia. Results were borderline significant in our dichotomized model for all cases and term preeclampsia. IL1β is a pro-inflammatory cytokine but our results do not support higher circulating levels of IL-1β in preeclampsia. This is consistent with a previous study of serum IL-1β at 34 weeks gestation [25]. This study suggested that reduced IL-1β may be a consequence of increased trophoblast debri based on a study reporting a downregulation of IL-1β by macrophages following phagocytosis of microvesicles in placentas from healthy women [35]. However, in another study examining placental microvesicles from preeclamptic women, there was significantly increased secretion of IL-1β compared to microvesicles from normal term placentas [36]. Placentas from preeclamptic women treated with MgSO4 prior to delivery, has been shown to increase IL-1β secretion compared to normal term placentas.[37] Thus, investigations at term may not represent systemic inflammation that occurs throughout pregnancy. IL-1β is suggested to be present in early pregnancy during implantation and may be necessary for immunotolerance at the maternal-fetal interface through modulation of the nuclear factor kappa-B pathway [38]. Therefore, it is possible that altered IL-1β expression may be associated with reduced maternal tolerance early in pregnancy. However, this may only be relevant to our first trimester samples. These findings need to be replicated in another large cohort with first and second trimester samples.

We found a significant association between detectable levels of TNFβ with all preeclampsia cases and term preeclampsia. Detectable IL-6 was also associated with term preeclampsia and had borderline significance with all preeclampsia cases. IL-6 has been associated with

preeclampsia in some [11, 12, 15, 17] but not all [8, 18, 19, 22] cross-sectional third trimester studies. In contrast, a nested case-control study of 71 women who developed subsequent preeclampsia and 71 matched (age, parity, and BMI) controls found no significant associations with IL-1β, IL-6, IL-10, TNF-α, or PAI-1 measured by ELISA at 18 weeks gestation [24]. Fewer studies have examined TNF-β. Although, TNF-β gene polymorphisms have been found to be associated with preeclampsia among European women [39] and have also been implicated to be associated with preeclampsia in incompatibility models [40].

TNF−β (also known as lymphotoxin), a pro-inflammatory cytokine part of the TNF superfamily [41], is expressed in higher concentrations in maternal plasma which is suggested to implicate local suppression of TNF-β during pregnancy rather than systemic down regulation [42]. IL6 is pleiotropic but also has pro-inflammatory functions and has been implicated in endothelium activation [43]. Our results suggest that when circulating TNF-β and IL6 are detectable they may increase the odds of preeclampsia. This would correspond with the hypothesis that preeclampsia is a state of heightened systemic inflammation [2]. However, our results were not consistent between statistical models. Furthermore, we found no significant associations with preterm preeclampsia. Both IL6 and TNFβ had a high percentage of non-detects in our population (>50%) which may lead to increased error when using models that impute non-detects by the LOD/2. The lack of association with preterm preeclampsia may be due to sample size and reduced power in that subgroup. Larger studies are needed to better understand whether there are differences in immune biomarkers between preeclampsia subgroups. As little is known about the role of TNF-β in preeclampsia, additional research is needed to further explore our observed association.

Major strengths of our study include the large sample size and blood sample collection prior to the third trimester of pregnancy. Limitations were our inability to assess inflammatory markers during the first trimester only and the use of a single measurement in each subject. Additionally, inclusion of women from Denmark may limit our generalizability to other populations. We did not have data on time of diagnosis of preeclampsia. Therefore, some women in our study may have had preeclampsia at the time of blood sampling. Although women in our study were sampled at a median of 16 weeks gestation we cannot rule out the presence of subclinical disease at the time of blood collection. Immune biomarker concentrations may vary by gestational age of sampling. However, we did not find significant differences in gestational age at sampling between cases and controls and inclusion into our models did not change our results. We did not have extensive data on infections or autoimmune diseases. We had access to a limited number of self-reported infections and autoimmune diseases but no significant associations were found with preeclampsia. However, self-reported data is often biased.

The in-house assay used in this study has been compared to other methods with biomarker measurements falling within similar ranges [44, 45]. However, there are no ‘‘true’’ concentrations for several immune biomarkers and results will vary by assay [46]. For example, antibodies from one immunoassay might bind to other epitopes than antibodies in another immunoassay. Because antibodies, matrixes, curve-fits and software vary between

methods, the levels of biomarker concentrations should not be directly compared [44]. Lastly, storing blood samples at room temperature may increase concentrations of some cytokines [47]. However, both cases and controls were stored in the same manner and should limit the potential for bias.

As immune dysregulation can be caused by several pathways (infection, obesity, etc.), future studies should focus on these pathways in relation to preeclampsia defined by subtype. This may provide insight into disease pathogenesis and perhaps aid in prediction model development. We found that as IL-1β levels increase there is a decrease in the log odds of preeclampsia, term and preterm preeclampsia. Detectable levels of IL6 and TNFβ were associated with preeclampsia and term preeclampsia in our dichotomized model. This may suggest that women with preeclampsia have a unique immune profile compared to normotensive women. However, we are unable to state whether these inflammatory markers are involved in the pathogenesis of preeclampsia and/or are markers of subclinical disease. Further, not all findings were consistent with different statistical approaches. All findings should be replicated in another large cohort. Consistent associations prior to preeclampsia diagnosis between independent studies may provide a better understanding of the mechanisms leading to preeclampsia subtypes. Further insight into preeclampsia pathogenesis is warranted before biomarkers with sufficient prediction value can be identified. Additionally, prediction will depend on evaluating more sensitive methods for biomarker detection that can be used in a clinical setting.

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Highlights

- We examined serum immune biomarkers in mid-pregnancy in relation to preeclampsia.
- This study included 706 participants of the Danish National Birth Cohort.
- We found that IL1β, IL6, IL4r and TNFβ were associated with preeclampsia, term preeclampsia and preterm preeclampsia, but were not consistent across statistical models.
- Future work focusing on clusters of immune biomarkers may be warranted.

Table 1

Comparison of baseline variables between women with preeclampsia and normotensive controls

	Normotensive N (%) N=297	**Preeclampsia N (%) N=409**	**Odds ratio (95% CI) or P-value**
Gestational week at sampling Mean(SD)	16.8±3.5	16.9±3.5	P=0.71
Maternal age (years)			
≥25	86 (29)	140 (34)	Reference
26–30	163 (55)	195 (48)	0.7 (0.5, 1.0)
31–35	43 (14)	62 (15)	0.9 (0.6, 1.4)
36+	5 (2)	12 (3)	1.5 (0.5, 4.3)
Body mass index			
<25	224 (77)	232 (58)	Reference
≥25 and <30	52 (18)	100 (25)	1.8 (1.2, 2.6)
≥30	16 (5)	65 (16)	3.8 (2.1, 6.8)
Smoking at enrollment			
No	256 (86)	376 (92)	Reference
Yes	41 (14)	33 (8)	0.6 (0.3, 0.9)

Logistic regression was used to calculated odds ratios, 95% confidence intervals and p-values

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Table 2

Mean, median and range of immune biomarkers and associations with preeclampsia

	Normotensive			Preeclampsia			
Biomarker	**Mean Pg/ml**	**Median Pg/ml**	**Range Pg/ml**	**Mean Pg/ml**	**Median Pg/ml**	**Range Pg/ml**	*** Effect estimate (p-value)**
RANTES	54.3	38.9	7.2–161.0	48.7	36.1	2.3–161.0	−0.13(0.0969)
IL4r	550.5	420.0	4.0–4000.0	454.9	373.5	4.0–4000.0	**−0.06(0.0383)**
IL6ra	121.2	114.9	28.9–400.00	121.6	112.6	20.1–400.0	−0.06(0.7282)
MIF	65.7	63.2	2.8–101.0	66.9	74.2	0.1–101.0	−0.06(0.3741)
MIP	706.6	685.0	10.0–10000.0	637.5	670.5	10.0–10000.0	−0.005(0.8710)
TNFR1	5.4	5.6	0.1–18.0	5.6	5.6	0.1–31.3	0.06(0.3007)
TNFα	97.6	4.0	4.0–4000.0	64.2	4.0	4.0–1770.0	**
IL6	140.0	4.0	4.0–4000.0	113.0	4.0	4.0–4000.0	0.03(0.4332)
IL4	70.7	21.0	4.0–2156.0	56.5	19.0	4.0–950.0	−0.02(0.6088)
IL5	44.7	27.0	4.0–1946.0	38.9	26.0	4.0–802.9	−0.02(0.7691)
IL12	185.9	4.0	4.0–4000.0	116.6	4.0	4.0–2764.0	−0.001(0.9775)
IL1α	31.8	4.0	4.0–611.0	20.9	4.0	4.0–627.4	**
IL1β	325.3	272.0	4.0–4000.0	296.3	214.5	4.0–4000.0	**−0.12(0.0068)**
IL8	168.2	4.0	4.0–4000.0	167.7	4.0	4.0–4000.0	0.01(0.7284)
IL10	325.3	252.0	4.0–4000.0	283.8	225.5	4.0–4000.0	−0.02(0.4864)
IFNγ	183.2	57.0	4.0–4000.0	190.2	112.5	4.0–3726.0	0.02(0.3738)
IL18	3.6	2.5	0.24–11.0	4.1	2.9	0.1–11.0	0.01(0.7284)
TGFβ	1479.4	821.0	39.0–13226.0	1557.7	1008.0	39.0–10256.0	0.04(0.1653)
TFNβ	657.3	499.0	10.0–6068.0	586.1	514.5	10.0–4398.0	0.04(0.1301)

* Immune markers were transformed to log (base 2) and adjusted for body mass index and smoking using logistic regression

** Comparisons were not made as > 75% of subjects measured beyond the LOD

Table 3

Detectable levels of immune biomarkers and the association with preeclampsia.

Biomarker	Normotensive N=297	Preeclampsia N=410	** Odds ratio (95% CI)
RANTES * n(%) detectable	265(89.2)	379(92.4)	0.7(0.4–1.2)
IL4r n(%) detectable	263(88.5)	345(84.2)	**0.7(0.4–1.0)**
IL6ra * n(%) detectable	294(98.9)	405(98.9)	0.8(0.2–3.7)
MIF * n(%) detectable	191(64.3)	254(62.0)	1.1(0.8–1.6)
MIP n(%) detectable	242(81.5)	338(82.4)	1.1(0.8–1.7)
TNFR1 n(%) detectable	269(90.6)	379(92.4)	1.3(0.8–2.3)
TNFα n(%) detectable	66(22.2)	105(25.6)	1.2(0.9–1.8)
IL6 n(%) detectable	81(27.3)	143(34.9)	**1.4(1.0, 2.0)**
IL4 n(%) detectable	163(54.9)	224(54.6)	1.0(0.7–1.4)
IL5 n(%) detectable	257(86.5)	368(89.8)	1.2(0.8–2.0)
IL12 n(%) detectable	133(44.7)	200(48.8)	1.2(0.9–1.6)
IL1α n(%) detectable	44(14.8)	67(16.3)	1.0(0.6–1.5)
IL1β n(%) detectable	280(94.3)	370(90.2)	**0.6(0.3, 1.0)**
IL8 n(%) detectable	98(33.0)	143(34.9)	1.1(0.8–1.6)
IL10 n(%) detectable	253(85.2)	342(83.4)	0.9(0.6–1.4)
IFNγ n(%) detectable	156(52.5)	231(56.3)	1.2(0.9–1.6)
IL18 * n(%) detectable	266(89.6)	359(87.6)	1.1(0.8–1.6)
TGFβ n(%) detectable	189(63.4)	286(69.8)	1.3(0.9–1.8)
TNFβ n(%) detectable	217(73.1)	326(79.5)	**1.6(1.1–2.3)**

** Immune markers were dichotomized by the LOD and adjusted for body mass index and smoking using logistic regression

* Indicates that biomarker had upper limit of detection (LOD)

Table 4

Median levels of immune biomarkers and the association with term and preterm preeclampsia (normotensive women are the reference).

	Preterm Preeclampsia		Term Preeclampsia	
Biomarker	**Median pg/ml (range)**	*** Effect estimate (p-value)**	**Median pg/ml (range)**	**** Effect estimate (p-value)**
RANTES	37.3(7.2–161.0)	−0.13(0.3323)	35.7(2.3–161.0)	−0.14(0.3323)
IL4r	353.0(4.0–4000.0)	−0.01(0.6410)	384.0(4.0–4000.0)	−0.01(0.0733)
IL6ra	113.0(20.1–280.2)	−0.01(0.7276)	111.8(29.4–400.00)	−0.05(0.7774)
MIF	63.1(0.58–101.0)	−0.20(0.0530)	78.3(0.1–101.0)	−0.02(0.8157)
MIP	599.6(10.0–1422.0)	−0.07(0.1403)	681.0(10.0–10000.0)	0.03(0.4135)
TNFR1	5.3(0.08–15.2)	−0.006(0.9206)	5.6(0.1–31.33)	0.1(0.1934)
TNFα	4.0(4.0–1770.0)	***	4.0(4.0–1550.0)	***
IL6	4.0(4.0–392.0)	−0.04(0.4585)	4.0(4.0–4000.0)	0.04(0.2267)
IL4	4.0(4.0–297.0)	−0.1(0.0578)	23.0(4.0–950.0)	0.01(0.8914)
IL5	25.0(4.0–213.0)	−0.05(0.5563)	26.0(4.0–802.9)	−0.007(0.9067)
IL12	4.0(4.0–932.0)	−0.01(0.3925)	6.0(4.0–2764.0)	0.01(0.7615)
IL1α	4.0(4.0–262.0)	***	4.0(4.0–627.4)	***
IL1β	199.0(4.0–985.0)	**−0.17(0.0065)**	229.0(4.0–4000.0)	**−0.10(0.0230)**
IL8	4.0(4.0–1160.0)	−0.1(0.2595)	4.0(4.0–4000.0)	0.02(0.4043)
IL10	230.0(4.0–989.0)	−0.04(0.4072)	225.0(4.0–4000.0)	−0.01(0.6078)
IFNγ	123.0(4.0–1455.0)	0.03(0.4557)	106.0(4.0–3726.0)	0.0(0.4390)
IL18	3.0(0.2–11.0)	−0.01(0.9633)	2.9(0.1–11.0)	0.13(0.0581)
TGFβ	740.0(39.0–5457.0)	−0.02(0.6847)	1061.0(39.0–10256.0)	0.06(0.0661)
TNFβ	370.0(10.0–2156.0)	−0.01(0.7571)	555.0(10.0–4398.0)	**0.1(0.0496)**

* Indicates that biomarker had upper limit of detection (LOD)

** Immune markers were transformed to log (base 2) and adjusted for body mass index and smoking using logistic regression. Normotensive women were the reference group.

*** Model 2 was not utilized as 75% of subjects measured beyond the LOD.

Table 5

Detectable levels of immune biomarkers and the association with term and preterm preeclampsia (normotensive women are the reference).

	Preterm Preeclampsia		Term Preeclampsia	
Biomarker	**n(%) detectable**	**** Odds ratio (95% CI)**	**n(%) detectable**	**** Odds ratio (95% CI)**
* RANTES	81(93.1)	0.6(0.3–1.6)	298(92.3)	0.7(0.4–1.2)
IL4r	73(83.9)	0.6(0.3–1.3)	272(84.2)	0.7(0.4–1.1)
* IL6ra	87(100.0)	***	318(98.5)	1.1(0.2–4.8)
* MIF2	59(67.8)	0.9(0.5–1.4)	195(60.4)	1.2(0.9–1.7)
MIP	64(73.6)	0.7(0.4–1.2)	274(84.8)	1.4(0.9–2.1)
TNFR1	79(90.8)	1.1(0.5–2.5)	300(92.9)	1.4(0.8–2.5)
TNFα	22(25.3)	1.2(0.7–2.2)	83(25.7)	1.2(0.9–1.8)
IL6	26(29.9)	1.1(0.7–1.9)	117(36.2)	**1.5(1.1–2.2)**
IL4	39(44.8)	0.7(0.4–1.1)	185(57.3)	1.1(0.8–1.5)
IL5	76(87.4)	1.0(0.5–2.0)	292(90.4)	1.3(0.8–2.2)
IL12	38(43.7)	1.0(0.6–1.6)	162(50.2)	1.3(0.9–1.8)
IL1α	9(10.3)	0.6(0.3–1.2)	58(17.9)	1.1(0.7–1.7)
IL1β	78(89.7)	0.5(0.2–1.2)	292(90.4)	**0.6(0.3–1.0)**
IL8	27(31.0)	1.0(0.6–1.6)	116(35.9)	1.2(0.8–1.6)
IL10	74(85.1)	1.0(0.5–2.0)	268(82.9)	0.9(0.6–1.4)
IFNγ	51(58.6)	1.3(0.8–2.1)	180(55.7)	1.1(0.8–1.6)
* IL18	81(93.1)	0.7(0.3–1.7)	278(86.1)	1.5(0.9–2.4)
TGFβ	54(62.1)	0.9(0.6–1.5)	232(71.8)	**1.4(1.0–2.0)**
TNFβ	66(75.9)	1.3(0.7–2.3)	260(80.5)	**1.7(1.1–2.5)**

* Indicates that biomarker had upper limit of detection (LOD)

** Immune markers were dichotomized by the LOD and adjusted for body mass index and smoking using logistic regression

*** Cell size too small for logistic regression