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Inflammatory Biomarkers and Spontaneous Preterm Birth Among Obese Women

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Abstract

Objective—To identify associations between second-trimester serum inflammatory biomarkers and preterm birth among obese women.

Methods—In this nested case-control study, we compared 65 serum inflammatory biomarkers in obese women whose pregnancies resulted in early spontaneous preterm birth (< 32 weeks gestation, n=34) to obese women whose pregnancies resulted in term birth (n=34). These women were selected from a larger population-based California cohort. Random forest and classification and regression tree techniques were employed to identify biomarkers of importance, and adjusted odds ratios (aORs) and 95% confidence intervals (CI) were estimated using logistic regression.

Results—Random forest and classification and regression tree techniques found that soluble vascular endothelial growth factor receptor-3 (sVEGFR3), soluble interleukin-2 receptor alpha-chain (sIL-2RA), and soluble tumor necrosis factor receptor 1 (sTNFR1) were related to preterm birth. Using multivariable logistic regression to compare preterm cases and term controls, decreased serum levels of sVEGFR3 and increased serum levels of sIL-2RA and sTNFR1 were associated with increased risk of preterm birth among obese women, aOR 3.2 (95% CI 1.0–9.9), aOR 2.8 (95% CI 0.9–9.0), and aOR 4.1 (95% CI 1.2 – 14.1), respectively.

Conclusions—In this pilot study, we identified three serum biomarkers indicative of inflammation to be associated with spontaneous preterm birth among obese women: sVEGFR3, sIL-2RA, and sTNFR1.

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Keywords

Inflammation; Preterm Birth; Inflammatory Markers; Obesity; vascular endothelial growth factor receptor-3; soluble interleukin-2 receptor alpha-chain; soluble tumor necrosis factor receptor I; vascular endothelial growth factor

INTRODUCTION

Globally, 15 million premature infants are born each year, with the highest rates in Africa and North America [1]. Thirteen percent of infants in the United States are born < 37 weeks gestation [2], the medical cost of which exceeds $26 billion per year [3]. Preterm birth (PTB) is a major cause of morbidity and mortality, and it accounts for approximately three fourths of all neonatal deaths [4, 5].

PTB is a complex phenomenon that is not well understood. Any disease process that induces myometrial contractility, cervical dilatation, and rupture of amniotic membranes—known as the “common pathway” of labor—prior to 37 weeks gestation can lead to spontaneous PTB [6]. Inflammatory cytokines and chemokines have been implicated in this “common pathway” and may mediate the association between certain disease states and PTB, such as intra-amniotic infection [7–11].

We recently reported an association between women’s pre-pregnancy obesity and early (<32 weeks gestation) PTB [12], which was consistent with earlier reports [13–14]. Since obesity itself is associated with chronic inflammation [15–17], we hypothesized that inflammatory processes may underlie the association between PTB and obesity.

In this nested case-control study, we examined biomarkers of inflammation in second-trimester serum samples, collected as part of routine screening for aneuoploidies and neural tube defects, in obese women who delivered prior to 32 weeks gestation compared to obese women who delivered at term.

METHODS

In this nested case-control study, we selected a subset of spontaneous PTB cases (< 32 weeks gestation, n=34) and term controls (≥ 37 weeks gestation, n=34) for comparison. All 68 case and control women were obese at the beginning of pregnancy, defined as body mass index (BMI) ≥ 30 kg/m^2, based on pre-pregnancy weight divided by height squared. These 68 obese women were selected from a larger cohort of 1000 women, who all had first and second trimester prenatal screening for aneuoploidies and neural tube defects through the California Genetic Disease Screening Program from July 2009 through December 2010. Details of the larger cohort have been described previously [18–25].

All collected samples were obtained at 15–20 weeks gestation and were stored by the California Biobank Program. Ultrasound dating was performed as part of routine prenatal screening. Demographic and obstetric information was collected from a linked hospital discharge birth cohort database maintained by the California Office of Statewide Health Planning and Development (OSHPD) that includes linked information from vital records and

hospital discharge records. From this dataset, the study included maternal pre-pregnancy BMI, age, race/ethnicity, education level, smoking, payer type, gestational age at delivery, parity, history of previous preterm birth, preexisting diabetes, gestational diabetes, preexisting hypertension, gestational hypertension, premature rupture of membranes, smoking, payer type, infant sex, gestational age at blood draw, and gestational age at delivery. Coding of diabetes, gestational diabetes, and hypertension was based on the International Classification of Diseases four digit codes contained in the cohort file.

Inflammatory biomarkers were measured in second-trimester serum samples. These samples were obtained from the California Biobank, where samples are stored in 1-mL tubes at −80°C until thawed for testing. In this study, all testing was performed by the Human Immune Monitoring Center (HIMC) at Stanford University. Serum testing of cytokines, chemokines, and soluble adhesion molecules was performed using a human 51-plex kit (Affymetrix Inc., Santa Clara, CA) (Figure 1, rows 1–4). Human soluble receptors were measured using a Millipore high sensitivity 14-plex kit (HSCRMAG32KPX14) (Billerica, MA) (Figure 1, row 5). Adiponectin was measured using a Millipore high sensitivity kit (HADK1MAG-61K) (Billerica, MA). The complete list of biomarkers is shown in Figure 1. All biomarkers were read per manufacturer recommendations using a Luminex 200 instrument (Austin, TX). Luminex lab protocols at HIMC have been described previously [26]. Median fluorescence intensity (MFI) values were reported for all markers using Masterplex software (Hitashi Solutions, San Bruno, CA). All analyses relied on the MFI average of two aliquots tested on the same plate for each case and control. All inter-assay coefficients were <15% across all markers and all intra-assay coefficients were <10%.

Statistical Analysis

Cases and controls were compared on all 65 analytes. Random forest, along with classification and regression tree (CART), techniques were employed to evaluate whether analytes differed between cases and controls. Random forest is a tree-based machine-learning algorithm that determines variable importance through a series of permutations and randomized node-optimizations. Random forest allows for examination of multiple factors simultaneously and accounts for interactions between those factors and non-linear associations with outcomes [27–28]. Party package in R software (Version 3.1.1) was used to run random forest. CART is also a machine-learning technique that we used (http://web.stanford.edu/~yesavage/ROC.html). Similar to random forest, CART identifies variables of relative importance in their contribution to the outcome of interest using recursive partitioning [29]. For CART, we did not perform replication owing to small sample sizes. For Random Forest to provide an interpretation of the best predictors, we calculated a variable importance measure for each potential predictor variable, using the “varimp ” function in the party package in R software, using the metric Mean Decrease Accuracy (MDA) [28]. To verify stability of measures, we did Jackknife sampling 100 times (randomly sampled 90% of the original data for 100 times) and calculated median and median absolute deviation of MDA out of 100 in permuted data, then compared the two ranking lists – MDA from original dataset, and median of MDA out of 100 permutated data.

Generalized additive regression models (GAM) with polynomial spline estimation were examined for each analyte to confirm data contour and suggested cutoffs values from CART outputs. Multivariable logistic regression was performed and adjusted odds ratios (aOR) and 95% confidence intervals (CI) were estimated for analytes identified as differing between cases and controls by random forest and CART, controlling for potential covariates (SAS 9.4, SAS Institute, Cary, NC).

Methods and protocols for the study were approved by the Committee for the Protection of Human Subjects within the Health and Human Services Agency of the State of California and the Institutional Review Board of Stanford University.

RESULTS

Demographic characteristics for PTB cases and term controls are shown in Table 1. Comparisons of the analytes between the two groups using means are shown in Table 2. There were no statistically significant differences in means (i.e., $p < 0.01$) between PTB cases and term controls. Random forest ranking plot identified three analytes of importance in sorting PTB cases from term controls: soluble vascular endothelial growth factor receptor 3 (sVEGFR3), soluble tumor necrosis factor receptor 1 (sTNFR1), and soluble interleukin-2 receptor alpha-chain (sIL-2RA) (Figure 2). CART decision tree analysis also identified sVEGFR3 and sIL-2RA as the most important markers. Suggested cutoff values were confirmed by spline estimation (Figure 3). The median value among term controls was used as the cutoff value for sTNFR1.

Logistic regression was performed for sVEGFR3, sIL-2RA, and sTNFR1. Among study women (all of whom were obese), serum sVEGFR3 < 23 (vs. ≥ 23), sIL-2RA ≥ 271.5 (vs. < 271.5), and sTNFR1 ≥ 733.0 (vs. < 733.0) were associated with PTB with aOR 3.2 (95% CI 1.0–9.9), aOR 2.8 (95% CI 0.9–9.0), and aOR 4.1 (95% CI 1.2 – 14.1), respectively, controlling for diabetes, hypertension, and parity. Patients with combined risk across multiple analytes were at higher risk of PTB, including sVEGFR3 < 23 and sIL-2RA ≥ 271.5 (aOR 8.9, 95% CI 1.7–47.3), sVEGFR3 < 23 and sTNFR1 ≥ 733.0 (aOR 52.6, 95% CI 3.6 – 764.2), and sTNFR1 ≥ 733.0 and sIL-2RA ≥ 271.5 (aOR 7.3, 95% CI 1.5 – 35.2), controlling for diabetes, hypertension, and parity.

DISCUSSION

In this study, we identified three inflammatory biomarkers—sVEGFR3, sIL-2RA, and sTNFR1—as being associated with spontaneous PTB among obese women in their mid-pregnancy serum. These findings are generally consistent with the hypothesis that inflammation may mediate the relationship between obesity and PTB, which we have described previously (Shaw 2014).

The group of vascular endothelial growth factor (VEGF) molecules (including VEGF, VEGFR1, VEGFR2, and VEGFR3) are important mediators of angiogenesis and direct growth of blood and lymphatic vasculature [30–31], and may contribute to fetal development and placental function [32–35]. Pathways related to angiogenesis have also been implicated in the etiology of preeclampsia [36–40]. VEGFR3 is a tyrosine kinase receptor expressed in

lymphatic vessels, and is activated by ligand VEGF-C, which promotes lymphangiogenesis [41–42]. Activation of this pathway has been associated with both inflammatory and anti-inflammatory processes [43–46].

The IL-2/IL-2RA (CD25) pathway is central to immune regulation [47]. Elevated levels of IL-2RA have been found in patients with infectious, inflammatory, and autoimmune diseases [48–53]. The TNF-α pathway is involved in systemic inflammation and has been implicated in both PTB and lipid metabolism [22, 54–58].

While a number of other studies have implicated other VEGF-related markers in PTB and preeclampsia, we know of no previous studies that have investigated the potential association between sVEGFR3, sIL-2RA, or sTNFR1 and PTB. However, there are biologically plausible hypotheses regarding these markers and PTB, although the differences in directionality between the three analytes and PTB risk remains puzzling. We believe that obesity may alter vascular growth and lymphangiogenesis of the fetal-placental unit, which may lead to failed tolerance and consequent inflammation. The signaling pathways related to maternal inflammation may impact placentation and increase risk of PTB. Our results are generally consistent with the “common pathway” theory of labor and PTB [6].

These observations should be interpreted with caution owing to the relatively small study sample size and the attendant multiple analyses conducted for this initial discovery approach. However, two different machine-learning analytic techniques, which are not affected by multiple testing, led us to focusing on the same two biomarkers.

Despite these limitations, we believe that these initial pilot findings are worthy of further pursuit. The interaction between obesity, PTB, and inflammatory biomarkers may lead to further understanding of the mechanism of PTB, and, potentially, to novel therapies targeted towards these underlying mechanisms.

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Abbreviations

PTB	Preterm birth
BMI	body mass index
HIMC	Human Immune Monitoring Center
MFI	median fluorescence intensity
CART	classification and regression tree
OR	odds ratio
CI	95% confidence interval

OSHPD	California Office of Statewide Health Planning and Development
sVEGFR3	vascular endothelial growth factor receptor-3
sTNFRI	soluble tumor necrosis factor receptor I
sIL2RA	soluble interleukin-2 receptor alpha-chain
VEGF	vascular endothelial growth factor

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Adipokines and Related Markers	Tumor necrosis factor-α (TNF-α), TNF-α super family members (CD40-ligand (CD40L), sFas Ligand (sFASL), TNF-β, TNF-related apoptosis-inducing ligand (TRAIL)), adiponectin, interleukin (IL)-1α, IL-6, leptin, plasminogen activator inhibitor-1 (PAI-1), resistin, adipose secreted chemokines (monocyte chemoattractant protein-1 (MCP-1), epithelial neutrophil-activating protein 78 (ENA-78))
Other Cytokines	Basic fibroblast growth factor (FGF-basic), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), IL-1α, IL-1β, IL-2, IL-2RA, IL-4, IL-5, IL-7, IL-8, IL-10, IL-15, IL-12p40, IL-12p70, IL-13, IL-17A, IL-17F, interferon α (IFN- α), IFN-β, IFN-γ, leukemia inhibitory factor (LIF), macrophage colony-stimulating factor (M-CSF), nerve growth factor (NGF), platelet-derived growth factor subunit BB (PDGF-BB, stem cell factor (SCF), transforming growth factor α (TGF-α), TGF-β, vascular endothelial growth factor (VEGF)
Other Chemokines	Eotaxin, growth-regulated protein α (GRO-α), IL-8, interferon-inducible protein-10 (IP-10), monocyte chemotactic protein-3 (MCP-3), monokine induced by gamma-interferon (MIG), macrophage inflammatory protein 1α (MIP-1α), MIP-1β, regulated on activation, normal T cell expressed and secreted (RANTES)
Soluble Adhesion Molecules	Soluble intercellular adhesion molecule -1 (s1CAM-1), vascular cell adhesion molecule-1 (sVCAM-1)
Soluble Receptors	TNF-α receptors (R) (TNFR1, TNFR2, CD30-ligand (CD30L)), IL-6 receptors (IL6R, GP130), IL-1 receptors (IL1R1, IL1R2), IL-1 receptor antagonist (RA) (IL-1RA), IL-4 receptor (IL4R), receptor for advanced glycosylation end products (RAGE), VEGF receptors (VEGFR1, VEGFR2, VEGFR3)

Figure 1.
List of serum biomarkers.

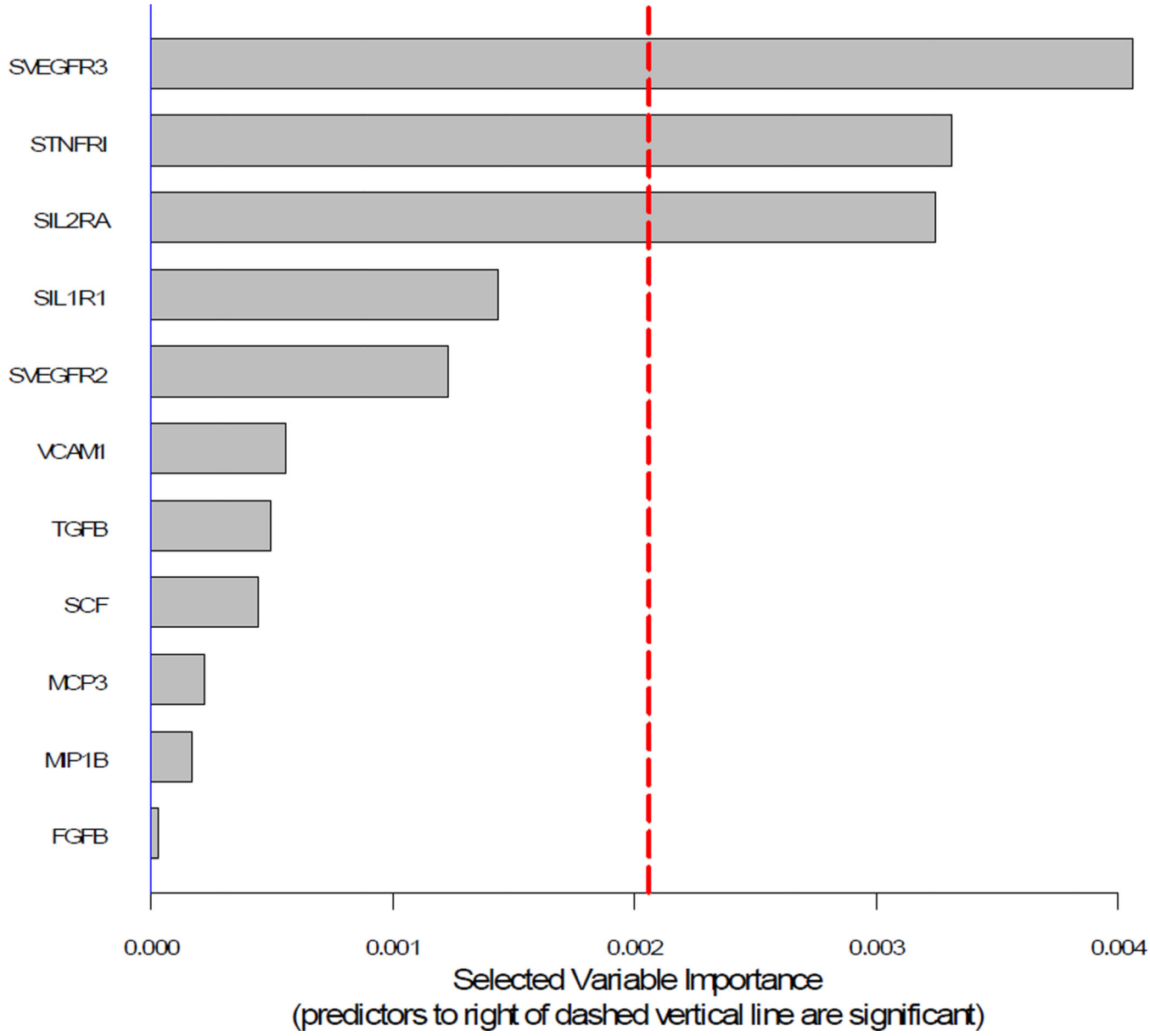

Figure 2.
Random forest ranking plot.

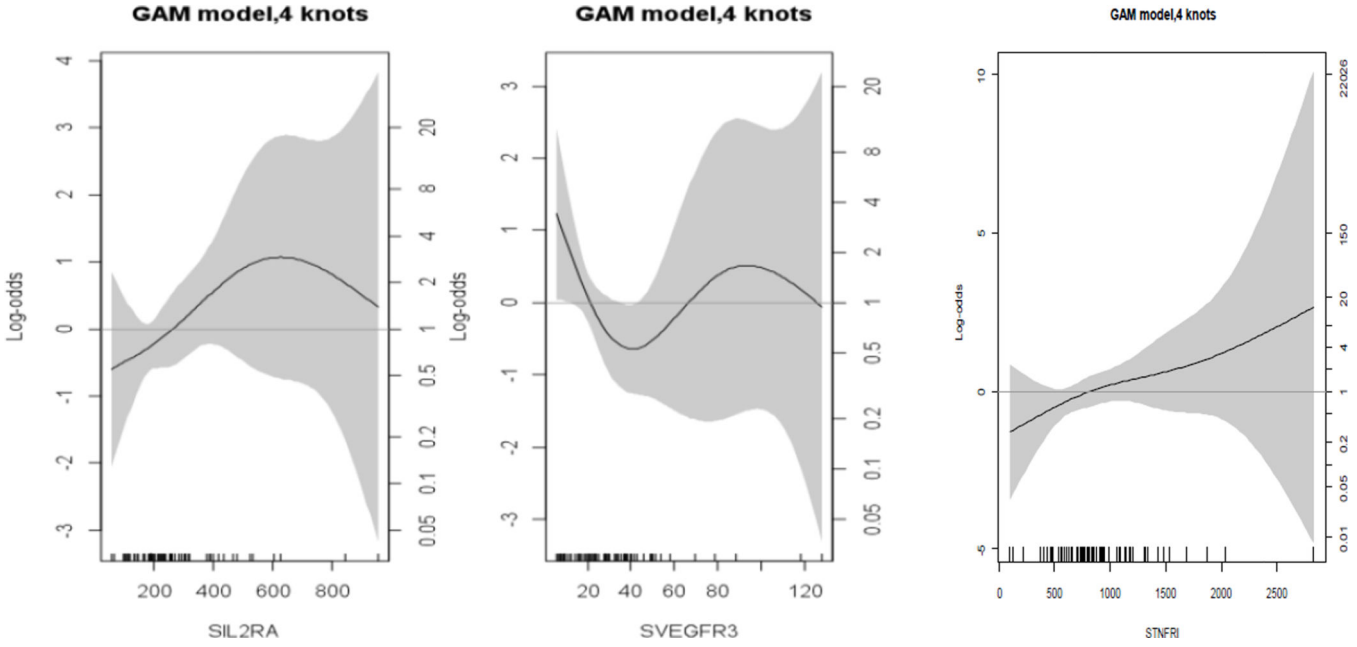

Figure 3.

Generalized additive regression models (GAM) with polynomial spline estimation for sIL2RA, sVEGFR3, and sTNFR1.

Table 1

Demographic characteristics of the preterm cases and term controls.

Variables	Preterm/obese (n=34) [1]	Term/obese (n=34) [1]
Age (years)		
<18	1 (2.9)	0
18–34	27 (79.4)	24 (70.6)
≥35	6 (17.7)	10 (29.4)
Race/Ethnicity		
Non-Hispanic White	11 (32.4)	11 (32.4)
Hispanic	21 (61.8)	21 (61.8)
Black	1 (2.9)	1 (2.9)
Asian/Other	1 (2.9)	1 (2.9)
Education		
Some high school	6 (17.7)	7 (20.6)
High school	13 (38.2)	9 (26.5)
Some college	7 (20.6)	11 (32.4)
≥4 year college degree	7 (20.6)	5 (14.7)
Prepregnancy BMI (kg/m^2)		
Obese I (30 – 34.9)	23 (67.7)	17 (50.0)
Obese II (35–39.9)	5 (14.7)	9 (26.5)
Obese III (≥40)	6 (17.7)	8 (23.5)
Parity		
1	18 (52.9)	7 (20.6)
≥2	16 (47.1)	27 (79.4)
Previous preterm[2]		
No	15 (93.8)	27 (100.0)
Yes	1 (6.3)	0
Any diabetes		
No	17 (50.0)	29 (85.3)
Yes	17 (50.0)	5 (14.7)
Any hypertension		
No	24 (70.6)	28 (82.4)
Yes	10 (29.4)	6 (17.7)
Any PROM[3]		
No	19 (55.9)	34 (100.0)
Yes	15 (44.1)	--
Any smoking		
No	32 (94.1)	34 (100.0)

Variables	Preterm/obese (n=34) [1]	Term/obese (n=34) [1]
Yes	2 (5.9)	0
Payer type for delivery		
Medi-Cal	22 (64.7)	23 (67.7)
Private	10 (29.4)	10 (29.4)
Self-pay	1 (2.9)	0
Other government	1 (2.9)	1 (2.9)
Infant sex		
Male	22 (64.7)	15 (44.1)
Female	12 (35.3)	19 (55.9)

[1] Obesity is defined as body mass index (BMI) ≥ 30. Numbers may not add to 100% due to rounding or missing values

[2] The denominator is those with parity ≥ 2

[3] Premature rupture of membranes (PROM)

Table 2

Comparisons of sample median fluorescence intensity (MFI) of biomarkers between the preterm cases and term controls using mean and standard deviation.

Variable	Term/Obese	Preterm/Obese	p-value
ADIPON	3216.6 (1776.9)	3021.9 (1535.8)	0.64
SCD30	15.3 (25.5)	10.8 (5.7)	0.08
SGP130	11082.5 (1326.0)	11037.1 (1513.6)	0.89
sIL-1R1	38.6 (9.3)	35.9 (9.8)	0.26
sIL-1RII	579.3 (349.2)	713.4 (393.6)	0.13
sIL-2RA	242.3 (149.4)	300.9 (180.0)	0.13
sIL-4R	106.0 (98.7)	112.5 (134.6)	0.80
sIL-6R	12599.2 (2328.9)	12576.3 (2193.6)	0.97
sRAGE	43.0 (24.4)	43.2 (24.9)	0.99
sTNFRI	778.9 (370.2)	997.1 (506.6)	0.04
sTNFRII	5221.9 (1248.5)	5252.7 (1797.2)	0.93
sVEGFR1	93.5 (39.4)	109.2 (74.4)	0.21
sVEGFR2	482.6 (108.1)	456.0 (110.3)	0.36
sVEGFR3	34.2 (22.4)	29.0 (24.6)	0.49
LEPTIN	8556.0 (2857.6)	8681.0 (3521.9)	0.85
SCF	290.2 (103.7)	277.1 (73.2)	0.46
MIG	409.2 (297.1)	376.8 (100.1)	0.33
MIP1-α	182.9 (93.9)	215.7 (353.9)	0.69
MCP3	274.1 (93.0)	286.6 (85.6)	0.55
PAI1	5357.7 (628.4)	5387.9 (674.4)	0.87
SFASL	397.7 (192.5)	378.3 (161.5)	0.60
ENA78	321.7 (370.3)	283.2 (321.8)	0.47
IL-1 β	292.0 (139.7)	383.1 (455.3)	0.74
IL-2	271.1 (112.5)	282.0 (105.3)	0.63
IL-4	243.9 (94.9)	239.8 (73.1)	0.80
IL-5	203.0 (98.4)	201.8 (75.9)	0.94
IP10	264.5 (100.3)	262.6 (68.1)	0.97
TGF-α	216.7 (81.6)	203.9 (70.4)	0.46
IL-6	126.2 (66.2)	159.0 (224.2)	0.32
IL-7	367.6 (199.9)	383.3 (164.5)	0.65
IL-8	2757.1 (2926.8)	3143.6 (3502.0)	0.64
IL-10	239.6 (93.3)	222.9 (58.7)	0.30
TGF-β	147.6 (59.5)	162.4 (70.3)	0.26
IFN--β	120.3 (53.3)	128.6 (70.9)	0.57
TNF-β	362.3 (149.9)	381.4 (150.6)	0.56
IL-12P40	792.2 (271.8)	756.9 (175.4)	0.51

Variable	Term/Obese	Preterm/Obese	p-value
IL-12P70	188.3 (75.8)	182.4 (53.9)	0.65
IL-13	104.2 (45.7)	98.7 (38.7)	0.53
IL-17	174.9 (72.5)	170.1 (58.6)	0.74
PDGF-BB	766.8 (423.7)	795.8 (434.2)	0.73
NGF	71.4 (31.1)	61.7 (16.7)	0.03
IL-17F	160.0 (65.7)	153.6 (62.8)	0.80
RANTES	12801.9 (1410.6)	12647.3 (1337.9)	0.67
IFN-γ	249.3 (85.4)	248.1 (71.8)	0.94
GMCSF	196.4 (70.2)	194.2 (64.0)	0.88
TNF-α	155.2 (65.3)	162.6 (96.7)	0.59
GCSF	64.3 (16.6)	61.1 (22.9)	0.43
MIP-1β	226.8 (89.6)	360.0 (874.8)	0.19
IFN-α	105.5 (43.0)	102.2 (41.6)	0.73
LIF	180.4 (80.6)	177.7 (68.4)	0.87
MCP1	724.1 (1244.6)	581.1 (424.9)	0.50
EOTAXI	226.0 (87.9)	224.6 (60.5)	0.93
FGFB	264.3 (105.3)	262.7 (89.5)	0.94
VEGF	134.8 (55.0)	131.3 (53.3)	0.79
TRAIL	185.6 (51.0)	181.8 (49.0)	0.76
GROA	252.2 (221.4)	280.7 (422.0)	0.65
IL-1α	196.0 (65.5)	183.1 (44.1)	0.29
IL-1RA	481.7 (734.2)	442.6 (514.8)	0.66
IL-15	123.8 (44.2)	120.6 (33.9)	0.74
ICAM1	7554.4 (4978.7)	6831.3 (4118.0)	0.42
HGF	535.2 (206.0)	514.3 (203.1)	0.61
CD40L	456.1 (485.7)	363.7 (378.1)	0.40
RESIST	4366.7 (1882.9)	4517.1 (1934.6)	0.74
VCAM1	15052.7 (2302.5)	13890.6 (3391.3)	0.07
MCSF	270.1 (108.4)	289.5 (128.8)	0.45