OBSTETRICS

Maternal biomarkers for fetal heart failure in fetuses with congenital heart defects or arrhythmias

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BACKGROUND: Diagnosis of fetal heart failure depends primarily on fetal ultrasonography assessment. Our recent study demonstrated that plasma natriuretic peptide levels in umbilical cord blood were correlated with the severity of heart failure in fetuses with congenital heart defects or arrhythmias. However, percutaneous umbilical blood sampling is an invasive procedure, and therefore, less or noninvasive biomarkers reflecting fetal heart failure are required.

OBJECTIVE: The aim of this study was to investigate the possibility of whether maternal serum biomarkers can diagnose fetal heart failure in fetuses with congenital heart defects or arrhythmias.

STUDY DESIGN: This exploratory cross-sectional study was conducted at a tertiary pediatric cardiac center. A total of 50 singletons with fetal congenital heart defects or arrhythmias and 50 controls who were registered in the National Cerebral and Cardiovascular Center Biobank from 2013 to 2016 were included. Maternal serum samples obtained during the third trimester were analyzed for 2 hormones and 36 cytokines using the Bio-Plex Pro Human Cancer Biomarker panels 1 and 2. We comprehensively analyzed the association between maternal serum biomarkers and ultrasonography findings or fetal arrhythmia status. Fetal heart failure was defined as a cardiovascular profile score $\leq$7.

RESULTS: Of 37 fetuses with congenital heart defects, heart failure was found in 1 case of tricuspid valve dysplasia with moderate tricuspid regurgitation. Of 13 fetuses with arrhythmias, 5 had heart failure at 28–33 weeks of gestation. Maternal serum cytokine and hormone concentrations were compared between patients with and without fetal heart failure at 28–33 weeks of gestation (n = 6 and n = 61, respectively). Sixty-one fetuses without heart failure consisted of 10 with congenital heart defect, 6 with arrhythmia, and 45 controls. Maternal serum concentrations of tumor necrosis factor-α, interleukin-6, soluble Fas ligand, transforming growth factor-α, and vascular endothelial growth factor-D were significantly higher when fetuses had heart failure than when they did not ($P < .05$), whereas maternal serum concentrations of heparin-binding epidermal growth factor-like growth factor were significantly lower when fetuses had heart failure than when they did not ($P < .05$). Multivariate analysis showed that maternal serum concentrations of tumor necrosis factor-α, vascular endothelial growth factor-D, and heparin-binding epidermal growth factor-like growth factor were independently associated with fetal heart failure. The cutoff values were as follows: tumor necrosis factor-α, 68 pg/mL (sensitivity of 50.0%, specificity of 93.4%, positive likelihood ratio of 7.6, negative likelihood ratio of 0.5); vascular endothelial growth factor-D, 1156 pg/mL (sensitivity of 50.0%, specificity of 93.4%, positive likelihood ratio of 7.6, negative likelihood ratio of 0.5); and heparin-binding epidermal growth factor-like growth factor, 90 pg/mL (sensitivity of 83.3%, specificity of 83.6%, positive likelihood ratio of 5.1, negative likelihood ratio of 0.2). The combination of these 3 cytokines showed sensitivity of 100%, specificity of 80.3%, positive likelihood ratio of 5.1, and negative likelihood ratio of 0. In the absence of fetal heart failure, concentrations of all maternal serum cytokines and hormones were similar in cases of fetal congenital heart defects and controls, while maternal serum soluble CD40 ligand concentrations were increased only in fetal arrhythmias.

CONCLUSION: Maternal serum concentrations of tumor necrosis factor-α, vascular endothelial growth factor-D, and heparin-binding epidermal growth factor-like growth factor were associated with fetal heart failure.

Key words: arrhythmia, biomarker, congenital heart defect, heart failure, prenatal diagnosis, proinflammatory cytokine

The diagnosis of fetal heart failure remains challenging and relies mainly on fetal ultrasonography assessment. Comprehensive assessments such as the cardiovascular profile (CVP) score have recently been used.[1] The CVP score may be useful in serial evaluations of fetuses at risk with myocardial dysfunction.[2]

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Our recent study demonstrated that plasma natriuretic peptide levels in umbilical cord blood were correlated with the severity of heart failure in fetuses with congenital heart defects (CHDs) or arrhythmias.[3] Percutaneous umbilical blood sampling is necessary to obtain real-time natriuretic peptide values, but because this is an invasive procedure, less invasive biomarkers reflecting fetal heart failure are required.

The fetus and mother are physiologically linked through the placenta. Mirror syndrome is an unusual pathological condition that manifests as maternal edema subsequent to severe fetal and placental hydrops.[4] Although its pathogenesis and pathophysiology remain unclear, it is possible that cytokines from hydropic placenta may increase maternal vasopermeability.[5] A previous study demonstrated high maternal plasma concentrations of soluble vascular endothelial growth factor receptor-1 (sVEGFR-1), an anti-angiogenic factor, in mirror syndrome.[6] Therefore, we hypothesized that maternal cytokine levels might reflect fetal heart failure caused by CHDs or arrhythmias.

The aim of this study was to investigate the possibility of whether maternal serum biomarkers can diagnose fetal heart failure in fetuses with CHDs

AJOG at a Glance

Why was this study conducted?

Because less or noninvasive biomarkers reflecting fetal heart failure are required, we investigated whether fetal heart failure could be diagnosed in the maternal circulation.

Key findings

Our study demonstrated that maternal serum concentrations of tumor necrosis factor-α, vascular endothelial growth factor-D, and heparin-binding epidermal growth factor-like growth factor were associated with fetal heart failure.

What does this add to what was known?

For the first time, we showed that maternal serum proinflammatory cytokines and apoptotic and angiogenic factors are potential candidates for predicting fetal heart failure in fetuses with congenital heart defects or arrhythmias.

or arrhythmias. We comprehensively analyzed the association between maternal serum concentrations of cytokines and both ultrasonography findings, quantified by the CVP score, and fetal arrhythmia status.

Materials and Methods

This single-center, exploratory, cross-sectional study was conducted with the approval of our institutional ethics board (M27-008). Written informed consent was obtained from all the patients.

Singletons prenatally diagnosed with fetal CHD or arrhythmia and registered in the National Cerebral and Cardiovascular Center (NCVC) Biobank between July 2013 and June 2016 were included in this study. Patients were excluded if their fetuses were diagnosed with critical chromosomal abnormalities, such as trisomy 13 or 18, or critical extracardiac anomalies requiring surgical intervention during the neonatal period.

Recruited controls were women whose fetuses had no morphological or chromosomal abnormalities and who were registered in the NCVC Biobank between July 2013 and June 2016. Cases and controls were selected in the order of registration in the NCVC Biobank. Exclusion criteria in both cases and controls also included maternal and obstetrical complications such as chronic hypertension, diabetes mellitus, preeclampsia, and gestational diabetes mellitus.

In the NCVC Biobank, maternal serum samples were routinely collected at 10−14 weeks, 28−33 weeks, and 34−39 weeks of gestation during pregnancy. However, maternal serum samples at 10−14 weeks of gestation were not usually available in patients with fetal CHD or arrhythmia because most of them were referred to the NCVC in the third trimester. Therefore, maternal serum samples at 28−33 weeks and 34−39 weeks of gestation were used in this study.

Our tertiary pediatric cardiac center has an established protocol that is implemented after a prenatal diagnosis of CHD or arrhythmia.[3,7] Outpatients with fetal CHD or arrhythmia were assessed biweekly by CVP score. Patients were admitted to the hospital and assessed at least weekly with CVP and biophysical profile scores after 37 weeks of gestation or if they have a complication such as threatened labor or fetal growth restriction (FGR). All diagnoses of CHD or arrhythmia were confirmed soon after birth by pediatric cardiologists.

In this study, the CVP score was used to assess fetal heart failure because it is not easy to distinguish types of fetal heart failure, given the fluctuating hemodynamics in various CHDs and arrhythmias.[1,8] Briefly, it is based on a composite scoring system to grade and serially follow the severity of fetal heart failure using 5 fetal echocardiographic parameters: fetal effusion, venous Doppler findings, heart size, cardiac function, and arterial Doppler findings (Table 1).

Heart failure severity is rated on a 10 point scale; points are deducted for abnormalities in each component marker. In this study because stratified analysis for severity of fetal heart failure was difficult because of the relatively small sample size, fetal heart failure was defined as a CVP score ≤7. A CVP score of 6 or 7 is considered to indicate moderate heart failure and ≤5 represents severe heart failure.[2,3] Moreover, fetal CHD and a CVP score ≤7 was at a risk of perinatal death.[8]

All fetal arrhythmias were diagnosed using fetal echocardiography (GE Medical Systems, Zipf, Austria) and magnetocardiography (MC-6400; Hitachi High-Technologies Corporation, Tokyo, Japan).[9] Fetal arrhythmias were categorized as tachyarrhythmias, bradyarrhythmias, or nonsustained extrasystoles. Fetal tachyarrhythmias, defined as a ventricular rate ≥180 beats per minute (bpm), included atrioventricular reentrant tachycardia, ectopic atrial tachycardia (EAT), and atrial flutter (AFL). When fetal tachyarrhythmia was sustained for ≥50% of the time on monitoring, fetal therapy using digoxin, sotalol, and flecainide was carried out.[10,11]

Fetal bradyarrhythmia consisted of atrioventricular block (AVB) and was defined as a ventricular rate <100 bpm. When fetal bradyarrhythmia was complicated with a fetal ventricular rate <55 bpm and myocarditis, beta-sympathomimetics and dexamethasone were used.[12,13]

Maternal serum samples were collected in the third trimester and at 1 week after delivery. After chilling maternal blood samples on ice, serum samples were prepared by centrifugation at 1500 × g for 15 min at 4°C and stored at −80°C until measurement. Serum samples were analyzed for 36 cytokines using the Bio-Plex Pro Human Cancer Biomarker Panels 1 and 2 (Bio-Rad, Hercules, CA) according to the manufacturer's protocol.[14] The threshold of each cytokine was routinely <5 pg/mL.

Human chorionic gonadotropin concentrations were measured using the AIA-PACK chemiluminescence

TABLE 1
Criteria for CVP score

Variables	Normal	−1 point	−2 points
Fetal effusion	Absence of effusion	Abdominal, pleural, or pericardial effusion	Skin edema
Venous Doppler finding	Normal Doppler	Reversed ductus venosus flow	Pulsatile flow in the umbilical vein
Heart size	CTAR <35%	CTAR between 35% and 50%	CTAR >50%
Cardiac function	Normal function	Holosystolic TR, or ventricular FS <28%	Holosystolic MR or CAVVR, or monophasic inflow pattern
Arterial Doppler finding	Normal Doppler	No end-diastolic UA flow	Reversed end-diastolic UA flow

The CVP score was calculated as follows: the maximum score for the 5 indices was 10, with points deducted according to disease severity. A CVP score of ≥8 is considered to indicate no or mild heart failure, 6 or 7 moderate heart failure, and ≤5 severe heart failure.

CAVVR, common atrioventricular valve regurgitation; *CHD*, congenital heart defect; *CTAR*, cardiothoracic area ratio; *CVP*, cardiovascular profile; *FS*, fractional shortening; *MR*, mitral valve regurgitation; *TR*, tricuspid valve regurgitation; *UA*, umbilical artery.

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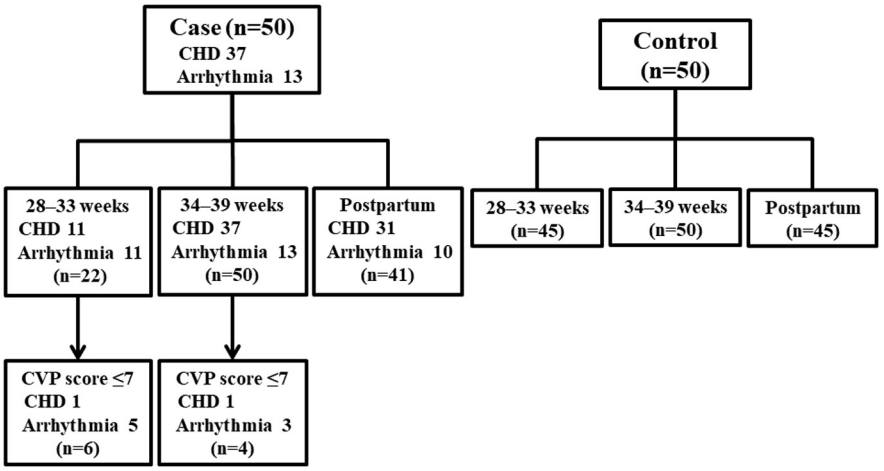
immunoassay (Tosoh Corporation, Tokyo, Japan). Alpha-fetoprotein concentrations were measured using chemiluminescence, with the human alpha-fetoprotein quantikine ELISA kit (R&D Systems, Minneapolis, MN).

Statistical analysis was performed using software Stata 14.1 (StataCorp LP, College Station, TX) and JMP 11 (SAS Institute, Cary, NC). Data are presented as means ± standard deviation or numbers of patients. All cytokine profiles were analyzed as continuous variables. A Student's *t* test or Wilcoxon signed-rank sum test was used to compare continuous variables between groups as appropriate. Categorical variables were evaluated using a Fisher exact test. Tukey-Kramer's honestly significant difference test was used to compare continuous variables among 3 or more groups.

We performed a principal component analysis to analyze the pattern of similarity between observations and the variables. We also conducted univariate and multivariate logistic analyses. The best prediction model was selected using stepwise backward elimination, with $P \geq .10$ as criterion for exclusion, which was adjusted for baseline variables. $P < .05$ was considered significant in all analyses.

A resampling analysis with 100 iterations was performed to identify the variables that entered into 50% of the logistic regression models to determine the independent predictor of SE (selection criterion: $P < .05$) using the mfp commands of Stata (StataCorp). Each cutoff value was calculated using receiver-operating characteristic (ROC) analysis. The area under the ROC curve of each variable was calculated using nonparametric analysis with bootstrap method.

FIGURE 1
Study flowchart



The term, 28—33 weeks, indicates cases in which maternal serum was available at 28—33 weeks of gestation. The term, 34—39 weeks, indicates cases in which maternal serum was available at 34—39 weeks of gestation. The term postpartum indicates the case in which maternal serum was available at 1 week after delivery.

CHD, congenital heart defect; *CVP*, cardiovascular profile.

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Results

Study cohort and baseline characteristics

A total of 50 women with fetal CHDs or arrhythmias and 50 controls were enrolled in the present study (Figure 1). All controls had a fetal CVP score of 10. Except for CVP scores, baseline perinatal characteristics were not significantly different between the 2 groups. Categories of CHDs and arrhythmias are shown in Table 2. Arrhythmias complicated by CHD were included in the arrhythmia group.

Of 37 fetuses with CHDs, 1 with tricuspid valve dysplasia and moderate tricuspid regurgitation had a CVP score of 7. One fetus with atrioventricular reentrant tachycardia, 5 with AFL, and 2 with EAT demonstrated cardioversion to sinus rhythm following fetal treatment. No fetus with complete AVB had an increase in CVP score in utero despite fetal

TABLE 2
Categories of CHDs and arrhythmias (n = 50)

Categories	CVP score ≤7	
	28–33 weeks of gestation	34–39 weeks of gestation
CHDs (n = 37)		
Isomerism (n = 7)		
Right atrial isomerism (n = 4)		
Left atrial isomerism (n = 3)		
Hypoplastic left heart syndrome (n = 5)		
Right heart defect (n = 6)		
Tricuspid valve dysplasia (n = 1)	1	1
Tricuspid atresia (n = 4)		
Double-inlet left ventricle (n = 1)		
Cyanotic heart defect (n = 12)		
Transposition of the great arteries (n = 5)		
Double-outlet right ventricle (n = 2)		
Tetralogy of Fallot (n = 3)		
Total anomalous pulmonary venous connection (n = 2)		
Acyanotic heart defect (n = 7)		
Coarctation of the aorta (n = 4)		
Atrioventricular septal defect (n = 2)		
Cardiac tumor (n = 1)		
Arrhythmias (n = 13)		
Tachyarrhythmia (n = 10)		
Atrioventricular reentrant tachycardia (n = 2)		
Atrial flutter (n = 5)	1	
Ectopic atrial tachycardia (n = 3)	2	
Complete atrioventricular block (n = 3)[a]	2	3

CHD, congenital heart defect; *CVP*, cardiovascular profile.

[a] One fetus with complete atrioventricular block had left atrial isomerism.

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treatment, resulting in a CVP score ≤7 at 34–39 weeks of gestation in all cases of complete AVB.

In summary, 1 fetus with AFL, 2 with EAT, and 2 with complete AVB had fetal heart failure at 28–33 weeks of gestation and 3 with complete AVB had heart failure at 34–39 weeks of gestation. One fetus with complete AVB had a CVP score of 5, and all others had a CVP score of 7. There were no cases of fetal hydrops or mirror syndrome.

Maternal serum biomarkers in cases with fetal heart failure (CVP score ≤7)

The median interval from blood sampling to freezing was 1 hour, but the interval ranged from 0 to 10 hours in the NCVC Biobank, so we investigated the coefficient of variation in the measured cytokine data for the serum samples processed up to 12 hours after blood sampling. This test of cytokine stability was performed in volunteers to minimize the burden on study participants. Serum samples from a volunteer pregnant woman and a volunteer adult man were prepared by centrifugation at 1, 2, 4, 6, and 12 hours after blood sampling. Using the 5 serum samples from each individual, we measured a series of cytokines and showed that the coefficient of variation was less than 10% for most cytokines in these serum samples (Supplemental Table 1).

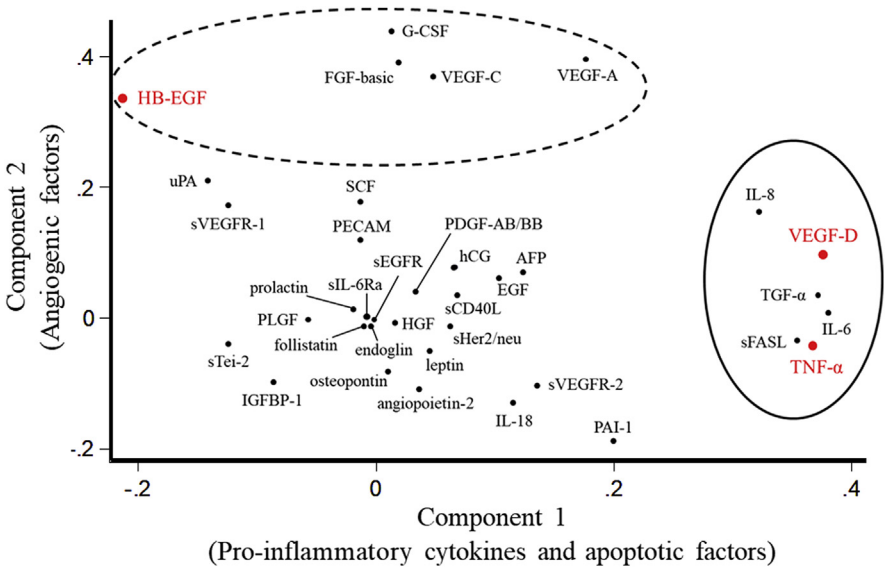
Maternal serum cytokine and hormone concentrations were compared between patients with and without fetal heart failure at 28–33 weeks of gestation (n = 6 and n = 61, respectively). Sixty-one fetuses without heart failure consisted of 10 with CHD, 6 with arrhythmia, and 45 controls. Baseline perinatal characteristics were similar in the 2 groups (Supplemental Table 2). All maternal examination data, such as peripheral blood, liver function, and renal function, were within normal range in both groups (Supplemental Table 3).

The results of principal component analysis are shown in Figure 2. The first component (25.5%) consisted of interleukin (IL)-6, IL-8, soluble Fas ligand (sFASL), transforming growth factor (TGF)-α, tumor necrosis factor (TNF)-α, and vascular endothelial growth factor (VEGF)-D, which are mainly proinflammatory cytokines and apoptotic factors. The second component (13.6%) consisted of fibroblast growth factor-basic, granulocyte-colony stimulating factor, heparin-binding epidermal growth factor-like growth factor (HB-EGF), VEGF-A, and VEGF-C, which are mainly angiogenic factors.

Maternal serum concentrations of TNF-α, IL-6, sFASL, TGF-α, and VEGF-D were significantly higher when fetal heart failure was present at 28–33 weeks of gestation than when it was not ($P < .05$) (Table 3). In contrast, maternal serum concentrations of HB-EGF were significantly lower when fetal heart failure was present at 28–33 weeks of gestation than when it was not ($P = .01$). Notably, all 6 of these cytokines were included in the first and second components.

Multivariate analysis showed that maternal serum concentrations of TNF-α (odds ratio [OR], 0.83; 95% confidence interval [CI], 0.70–0.99),

FIGURE 2
Principal component analysis of maternal serum cytokine and hormone concentrations at 28–33 weeks of gestation



To determine which of the original 2 hormones and 36 cytokines were most important, we conducted a principal component analysis, which transforms a number of possibly correlated variables into a smaller number of uncorrelated variables. The first component (25.5%, *solid circle*) consists of IL-6, IL-8, sFASL, TGF-α, TNF-α, and VEGF-D, which are mainly proinflammatory cytokines and apoptotic factors. The second component (13.6%, *dotted circle*) consists of FGF-basic, G-CSF, HB-EGF, VEGF-A, and VEGF-C, which are mainly angiogenic factors.

AFP, alpha-fetoprotein; *EGF*, epidermal growth factor; *FGF*, fibroblast growth factor; *G-CSF*, granulocyte-colony stimulating factor; *HB-EGF*, heparin-binding epidermal growth factor-like growth factor; *hCG*, human chorionic gonadotropin; *HGF*, hepatocyte growth factor; *IGFBP*, insulin-like growth factor binding protein; *IL*, interleukin; *PAI*, plasminogen activator inhibitor; *PDGF*, platelet-derived growth factor; *PECAM*, platelet and endothelial cell adhesion molecule; *PLGF*, placental growth factor; *sCD40L*, soluble CD40 ligand; *SCF*, stem cell factor; *sEGFR*, soluble epidermal growth factor receptor; *sFASL*, soluble Fas ligand; *sHER*, soluble human epidermal growth factor receptor; *sVEGFR*, soluble vascular endothelial growth factor receptor; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor; *uPA*, urokinase-type plasminogen activator; *VEGF*, vascular endothelial growth factor.

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VEGF-D (OR, 1.03; 95% CI, 1.00–1.05), and HB-EGF (OR, 0.95; 95% CI, 0.92–0.99) were associated with fetal heart failure (Table 4). The cutoff values calculated using ROC analysis were as follows (Supplemental Figure); TNF-α, 68 pg/mL; VEGF-D, 1156 pg/mL; and HB-EGF, 90 pg/mL. Sensitivity, specificity, likelihood ratio, positive predict value, and negative predict value in addition to the area under the ROC curve of each maternal serum cytokine and their combination in fetal heart failure are shown in Table 5.

TABLE 3
Maternal serum concentrations of cytokines in cases with and without fetal heart failure at 28–33 weeks of gestation (n = 67)

Variables	CVP score ≥8 (n = 61)	CVP score ≤7 (n = 6)	*P* value
TNF-α, pg/mL	33.1 ± 14.7	50.2 ± 38.7	.03
IL-6, pg/mL	59.7 ± 23.1	82.4 ± 48.9	.04
sFASL, pg/mL	367.5 ± 141.8	539.0 ± 346.2	.02
TGF-α, pg/mL	86.3 ± 28.9	119.7 ± 66.4	.02
VEGF-D, pg/mL	989.7 ± 108.2	1101.5 ± 201.5	.03
HB-EGF, pg/mL	183.0 ± 45.2	128.0 ± 47.9	.01

Data are presented as mean ± SD. $P < .05$ indicates a significant difference.

CVP, cardiovascular profile; *HB-EGF*, heparin-binding epidermal growth factor-like growth factor; *IL*, interleukin; *sFASL*, soluble Fas ligand; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor; *VEGF*, vascular endothelial growth factor.

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Maternal serum biomarkers in cases without fetal heart failure (CVP score ≥8)

In women without fetal heart failure at 28–33 weeks of gestation, maternal serum cytokine and hormone concentrations were compared among cases of fetal CHD (n = 10), cases of fetal arrhythmia (n = 6), and controls (n = 45). Maternal serum concentrations of soluble human epidermal growth factor receptor 2/neu, soluble CD40 ligand (sCD40L), and plasminogen activator inhibitor-1 were significantly higher in cases of fetal arrhythmia than in cases of fetal CHD ($P < .05$). Maternal serum HB-EGF concentrations were significantly lower in cases of fetal arrhythmia than in cases of fetal CHD ($P = .02$). Maternal serum concentrations of soluble human epidermal growth factor receptor 2/neu and sCD40L were significantly higher in cases of fetal arrhythmia than in controls ($P < .05$).

Additionally, in women without fetal heart failure at 34–39 weeks of gestation, maternal serum cytokine and hormone concentrations were compared among cases of fetal CHD (n = 36), cases of fetal arrhythmia (n = 10), and controls (n = 50). Maternal serum concentrations of sCD40L and VEGF-D were significantly higher in cases of fetal arrhythmia than cases of fetal CHD ($P < .05$). Maternal serum concentrations of soluble Tei-2, sCD40L, and TGF-α were significantly higher in cases of fetal arrhythmia than in controls ($P < .05$).

Collectively, these data indicated that maternal serum sCD40L concentrations were significantly higher in cases of fetal arrhythmia than in cases of fetal CHD and controls, both at 28–33 and 34–39 weeks of gestation in the absence of fetal heart failure ($P < .05$) (Figure 3, A and B). Conversely, in cases of fetal CHD without heart failure, all maternal serum cytokine and

TABLE 4
Univariate and multivariate analyses of maternal serum concentrations in fetal heart failure

	Univariate			Multivariate[a]			Bootstrap validation reliability, %[b]
Variables	OR	95% CI	*P* value	OR	95% CI	*P* value	
TNF-α	1.04	1.00–1.07	.05	0.83	0.70–0.99	.04	60
IL-6	1.02	1.00–1.05	.07				38
sFASL	1.00	1.00–1.01	.04				26
TGF-α	1.02	1.00–1.04	.04				30
VEGF-D	1.01	1.00–1.01	.04	1.03	1.00–1.05	.04	54
HB-EGF	0.98	0.96–1.00	.02	0.95	0.92–0.99	.01	76

CI, confidence interval; *CVP* score, cardiovascular profile score; *HB-EGF*, heparin-binding epidermal growth factor-like growth factor; *IL*, interleukin; *OR*, odds ratio; *sFASL*, soluble Fas ligand; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor; *VEGF*, vascular endothelial growth factor.

[a] The best prediction model was selected using stepwise backward elimination with $P \geq .10$ as the criterion for exclusion, which was adjusted for baseline variables. $P < .05$ indicates a significant difference; [b] Percent (%) refers to the reliability determined by the bootstrap validation method. A resampling analysis with 100 iterations was performed to identify the variables that were included in 50% of the logistics regression models.

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hormone concentrations were similar to controls both at 28–33 and 34–39 weeks of gestation.

Maternal serum biomarkers during pregnancy and postpartum in controls

Trends in maternal cytokine and hormone concentrations during pregnancy and postpartum in controls are shown in Supplemental Table 4. During pregnancy compared with postpartum, TNF-α, sFASL, and TGF-α levels were higher, HB-EGF and sCD40L levels were lower, and IL-6 and VEGF-D levels were not significantly different.

Comment

Principal findings

Our study demonstrated that maternal serum concentrations of TNF-α, VEGF-D, and HB-EGF were associated with fetal heart failure in fetuses with CHDs or arrhythmias. Furthermore, in fetuses without heart failure, all maternal serum cytokine and hormone concentrations were similar between cases of fetal CHD and controls, while maternal serum sCD40L concentrations were increased in cases of fetal arrhythmia.

Clinical implications

Maternal serum TNF-α concentrations were associated with fetal heart failure. TNF-α is a well-known proinflammatory cytokine that is present at sites of inflammation.[15] Our results suggested that fetal heart failure may be associated with the maternal response to systemic inflammation.

The physiological states of the fetus and mother are closely linked via the placenta. Mirror syndrome, manifesting as maternal pulmonary and skin edema, occurs when fetal heart failure causes both fetal and placental hydrops.[4] Although its pathogenesis and pathophysiology remain unclear, it is possible that cytokines from a hydropic placenta with trophoblastic damage may increase maternal vasopermeability.[5]

Our recent study demonstrated that plasma natriuretic peptide levels in umbilical cord blood were correlated with the severity of fetal heart failure.[3] Elevated natriuretic peptide levels are mainly attributable to increases in

TABLE 5
Comparison of the AUCs for maternal serum cytokines and their combinations in fetal heart failure

Variables	Sensitivity	Specificity	Positive likelihood ratio	Negative likelihood ratio	PPV	NPV	AUC	95% CI
TNF-α	50.0	93.4	7.63	0.54	42.9	95.0	0.50	0.08–0.92
VEGF-D	50.0	93.4	7.63	0.54	42.9	95.0	0.62	0.30–0.94
HB-EGF	83.3	83.6	5.08	0.20	33.3	98.1	0.82	0.68–0.97
TNF-α plus VEGF-D	50.0	93.4	7.63	0.54	42.9	95.0	0.62	0.30–0.94
TNF-α plus HB-EGF	83.3	83.6	5.08	0.20	33.3	98.1	0.83	0.69–0.96
VEGF-D plus HB-EGF	83.3	83.6	5.08	0.20	33.3	98.1	0.84	0.70–0.99
TNF-α plus VEGF-D plus HB-EGF	100	80.3	5.08	0.00	33.3	100	0.90	0.81–0.98

Each cutoff value was calculated using ROC analysis: TNF-α, 68 pg/mL; VEGF-D, 1156 pg/mL; and HB-EGF, 90 pg/mL. Using nonparametric analysis of ROC curve under covariates with the bootstrap method, each AUC was calculated, and then the AUCs for maternal serum cytokines and their combinations were not significantly different compared with the AUC for TNF-α plus VEGF-D plus HB-EGF.

AUC, area under the ROC curve; *CI*, confidence interval; *HB-EGF*, heparin-binding epidermal growth factor-like growth factor; *NPV*, negative predictive value; *PPV*, positive predictive value; *ROC*, receiver operating characteristic; *TNF*, tumor necrosis factor; *VEGF*, vascular endothelial growth factor.

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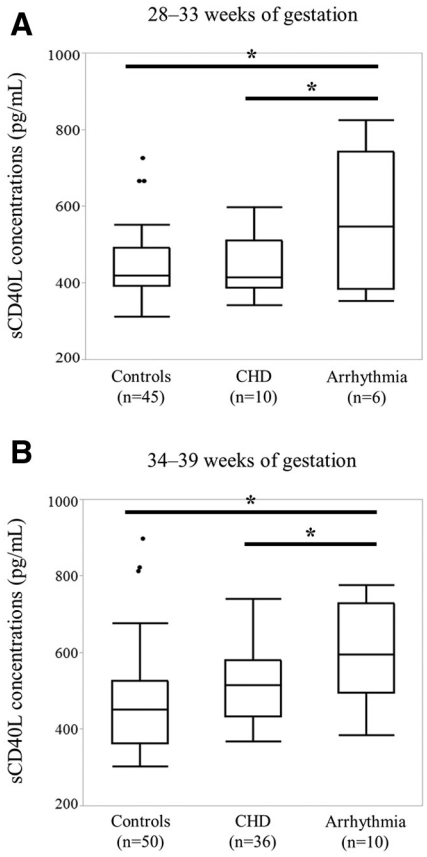
FIGURE 3
Maternal serum sCD40L concentrations



Maternal serum sCD40L concentrations are significantly higher in cases of fetal arrhythmia than in cases of fetal CHD and in controls at 28—33 weeks of gestation **(A)** and at 34—39 weeks of gestation **(B)**, respectively. *Boxes* indicate median and IQR, *whiskers* are range excluding outliers more than 1.5 × IQR from upper or lower quartile, and *circles* are outliers. The asterisk indicates the Tukey-Kramer honest significant difference test that was used ($P < .05$ indicates a significant difference).

CHD, congenital heart defect; *IQR*, interquartile range; *sCD40L*, soluble CD40 ligand.

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central venous pressure secondary to arrhythmias or CHD-induced atrioventricular valve regurgitation. Thus, we speculated that increased central venous pressure in fetal heart failure may damage placental trophoblasts and increase proinflammatory cytokines, resulting in the elevation of both maternal and placental vasopermeability.[5]

Espinoza et al[6] reported that high maternal sVEGFR-1 concentrations were observed in mirror syndrome. Excess sVEGFR-1 binds to and inactivates VEGF and thereby induces systemic endothelial dysfunction.[16] In this study, fetal heart failure did not induce high sVEGFR-1 and low VEGF levels in maternal serum, but it was associated with maternal systemic inflammatory cytokines such as IL-6 and TNF-α. Therefore, in the future, it is necessary to measure and compare the changes of maternal serum cytokines in patients with more severe fetal heart failure and mirror syndrome.

Maternal serum concentrations of VEGF-D and HB-EGF were also associated with fetal heart failure in this study. VEGF-D and HB-EGF are known to be angiogenic factors that are closely correlated with organ injury and repair. Cells in inflammatory tissue are often damaged and need repair. Maternal concentrations of these cytokines may be altered secondary to trophoblast injury that results in inflammation.

Llurba et al[17] found that an intrinsically angiogenic impairment exists in CHD that appears to be present in both the maternal and fetal circulations. The authors suggested that an imbalance of angiogenic-antiangiogenic factors is associated with developmental defects of the human heart. However, in this study, women whose fetuses had CHD but no heart failure demonstrated that serum concentrations of cytokines, including angiogenic factors, were similar to those of controls. Thus, our results indicate that maternal serum cytokine levels are altered by fetal heart failure rather than by morphological abnormalities of the fetal heart.

In this study, maternal serum sCD40L concentrations were associated with fetal arrhythmias in cases in which the fetuses did not have heart failure. sCD40L is a transmembrane protein that is shed from activated platelets and is involved in the activation of endothelial cells.

Inwald et al[18] found that the binding of sCD40L to platelet CD40L caused CD62P expression and that the release of the contents of α-granules and dense granules participated in the recruitment and activation of leukocytes. However, Henn et al[19] reported that even high levels of sCD40L failed to induce an inflammatory reaction. They concluded that the CD40L-CD40 interaction eventually transforms CD40L to the biologically inactive sCD40L, thereby effectively limiting the inflammatory process in the vascular system. Thus, data on whether sCD40L is capable of eliciting an inflammatory response remain controversial.

Our results showed that in women without fetal heart failure, serum sCD40L concentrations were significantly higher in cases of fetal arrhythmia than in cases of fetal CHD or controls. Even when fetal therapy is not indicated, both fetal tachyarrhythmia and bradyarrhythmia in the absence of heart failure may damage trophoblasts and alter endothelium-platelet interactions.

Study strengths and limitations

The present study had several strengths. First, this was the first study to demonstrate that maternal serum cytokine concentrations may indicate the presence of fetal heart failure. Interestingly, maternal serum cytokine levels were affected not by morphological abnormalities of the developing heart but by fetal heart failure secondary either to an arrhythmia or to CHD-induced atrioventricular valve regurgitation. Second, our center is one of the largest tertiary pediatric cardiac institutions in Japan, and women whose fetuses had a variety of complex CHDs and arrhythmias were included in the study cohort. In addition, all fetuses with CHDs or arrhythmias were diagnosed prenatally with high accuracy, and serial assessment using the CVP score was available. Thus, we were able to unambiguously investigate the relationship between maternal serum cytokine levels and cases of fetal heart failure with identified pathoetiologies.

There were several limitations to the present study, beyond its exploratory nature. First, only the Bio-Plex Pro Human Cancer Biomarker panels 1 and 2 was used to assess the cytokines in this study, and

therefore, it is unclear whether other cytokines can predict fetal heart failure. Second, the most severe cases, namely fetal hydrops or mirror syndrome, were not included in the analysis, partially because of the efficacy of transplacental therapy for fetal arrhythmias. Third, it is difficult to evaluate the correlation between maternal serum biomarker levels and the severity of fetal heart failure or types of CHD and arrhythmia because of the relatively small sample size. Lastly, we have not yet clarified whether maternal serum biomarkers are useful for assessing fetal heart failure prior to the third trimester. Even in normal pregnant women, maternal serum concentrations of some cytokines, such as sVEGFR-1 and placental growth factor, fluctuate during pregnancy.[20,21]

Maternal characteristics and conditions may also influence maternal serum cytokine levels. Therefore, to minimize the biases in this study cohort, we focused on the third trimester and excluded cases with maternal and obstetrical complications.

Conclusions and future research direction

Our study demonstrated that maternal serum concentrations of TNF-α, VEGF-D, and HB-EGF were associated with fetal heart failure. For the first time, we showed that maternal serum proinflammatory cytokines and apoptotic and angiogenic factors are potential candidates for predicting fetal heart failure in fetuses with CHDs or arrhythmias. In the future, larger multicenter prospective cohort studies are needed to clarify whether maternal serum concentrations of TNF-α, VEGF-D, and HB-EGF can assess the severity of heart failure and determine the efficacy of fetal therapy.

As a general issue, the most frequent cause of fetal heart failure is FGR due to placental insufficiency.[22] The pathophysiology of fetal cardiac failure is quite different between FGR on the one hand and CHD or arrhythmia on the other. Thus, it is beneficial to investigate whether heart failure has different maternal biomarker signatures, depending on whether it is caused by FGR vs CHD or arrhythmia. We believe that our results will help optimize the design of future studies to accurately determine whether maternal serum biomarkers can predict fetal heart failure. ■

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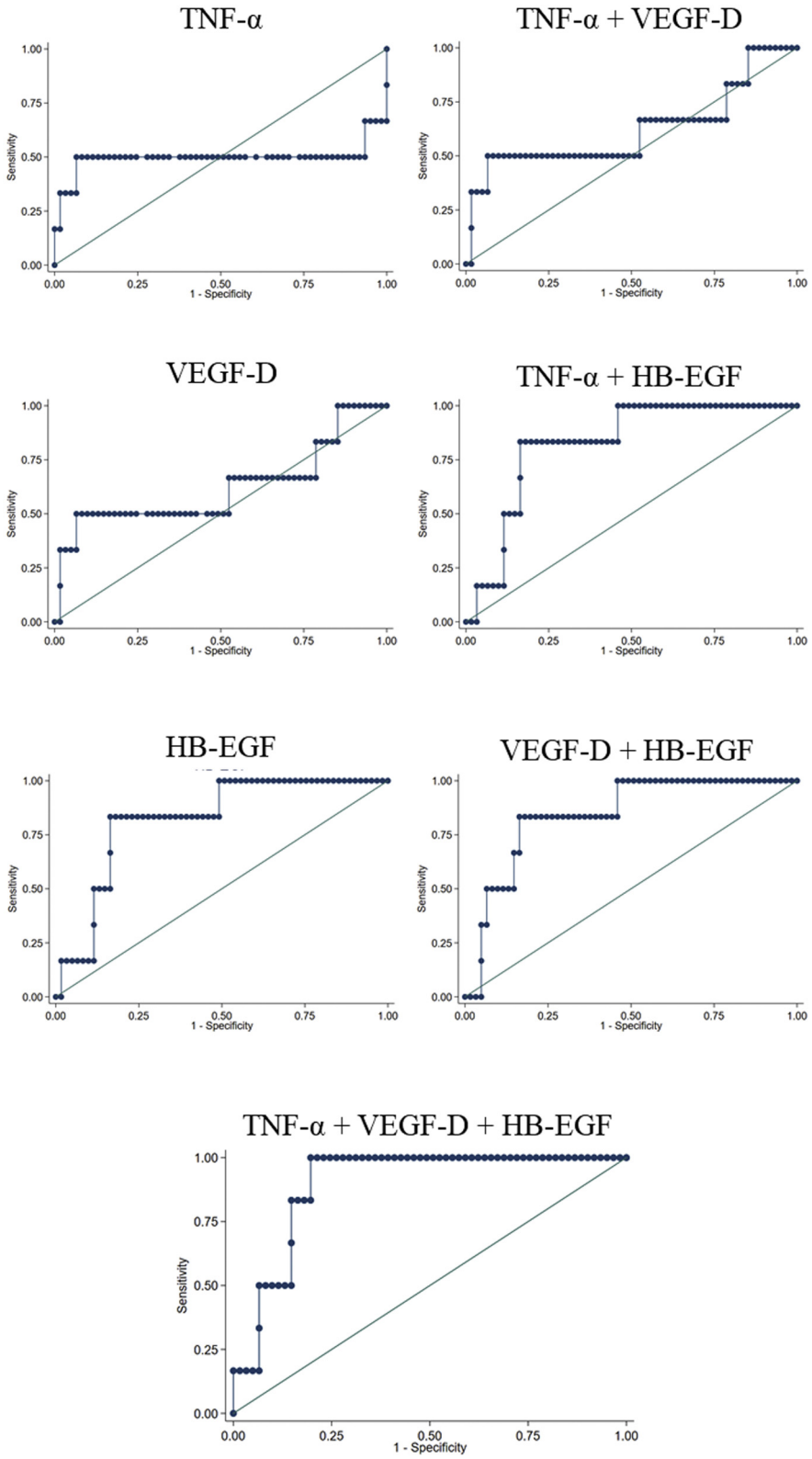
SUPPLEMENTAL FIGURE
ROC curves of maternal serum cytokines and combination in FHF



The ROC curves of each maternal serum cytokine and their combination in fetal heart failure are shown.

FHF, fetal heart failure; *HB-EGF*, heparin-binding epidermal growth factor-like growth factor; *ROC*, receiver operating characteristic; *TNF*, tumor necrosis factor; *VEGF*, vascular endothelial growth factor.

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SUPPLEMENTAL TABLE 1

Coefficient of variation of each cytokine concentration in serum samples from 2 volunteers: a pregnant woman and an adult man

Variables	Pregnant woman	Adult man
sEGFR	5.0	3.2
FGF-basic	0.9	5.6
Follistatin	4.3	13.9
G-CSF	2.0	12.1
sHER2/neu	5.7	7.0
HGF	4.0	6.4
sIL-6Ra	6.4	6.2
Leptin	7.7	10.9
Osteopontin	10.8	7.2
PDGF-AB/BB	5.6	4.3
PECAM-1	3.1	3.2
Prolactin	5.8	3.5
SCF	3.0	7.6
sTIE-2	5.5	8.5
sVEGFR-1	11.8	7.9
sVEGFR-2	5.5	4.4
Angiopoietin-2	12.0	14.8
sCD40L	12.3	8.5
EGF	18.0	19.5
Endoglin	3.1	7.2
sFASL	5.7	8.5
HB-EGF	5.6	8.9
IGFBP-1	8.1	10.2

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SUPPLEMENTAL TABLE 1

Coefficient of variation of each cytokine concentration in serum samples from 2 volunteers: a pregnant woman and an adult man *(continued)*

Variables	Pregnant woman	Adult man
IL-6	7.5	8.1
IL-8	3.1	10.1
IL-18	2.8	2.0
PAI-1	5.0	8.0
PLGF	3.9	7.9
TGF-a	7.6	9.9
TNF-a	5.9	10.0
uPA	5.1	12.3
VEGF-A	5.0	6.7
VEGF-C	4.5	9.9
VEGF-D	2.9	6.3

Serum samples from a volunteer pregnant woman and a volunteer adult man were prepared by centrifugation at 1, 2, 4, 6, and 12 hours after blood sampling and stored at −80°C until measurement. The coefficient of variation of each cytokine concentration was calculated using the 5 serum samples from each individual.

EGF, epidermal growth factor; *FGF*, fibroblast growth factor; *G-CSF*, granulocyte-colony stimulating factor; *HB-EGF*, heparin-binding epidermal growth factor-like growth factor; *HGF*, hepatocyte growth factor; *IGFBP*, insulin-like growth factor binding protein; *IL*, interleukin; *PAI*, plasminogen activator inhibitor; *PDGF*, platelet-derived growth factor; *PECAM*, platelet and endothelial cell adhesion molecule; *PLGF*, placental growth factor; *sCD40L*, soluble CD40 ligand; *SCF*, stem cell factor; *sEGFR*, soluble epidermal growth factor receptor; *sFASL*, soluble Fas ligand; *sHER*, soluble human epidermal growth factor receptor; *sVEGFR*, soluble vascular endothelial growth factor receptor; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor; *uPA*, urokinase-type plasminogen activator; *VEGF*, vascular endothelial growth factor.

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SUPPLEMENTAL TABLE 2

Baseline perinatal characteristics in patients with and without fetal heart failure at 28–33 weeks of gestation (n = 67)

Characteristics	CVP score ≥8 (n = 61)	CVP score ≤7 (n = 6)	*P* value
Maternal age, y	33.6 ± 4.9	31.6 ± 2.9	.36
Assisted reproductive technology	8 (13.1)	0	1.00
Primipara	27 (44.3)	3 (50.0)	1.00
Body mass index ≥25 kg/m^2	1 (1.6)	0	1.00
Smoking	1 (1.6)	0	1.00
Male infant	30 (49.2)	2 (33.3)	.66
Chromosomal abnormality	1 (1.6)	0	1.00
Fetal growth restriction	3 (4.9)	1 (16.7)	.32
Gestational age at delivery, wks	38.4 ± 1.3	37.6 ± 0.5	.15
Birthweight, g	2918 ± 350	2833 ± 380	.61
Apgar score <7 at 5 min	1 (1.6)	0	1.00
Umbilical cord arterial pH <7.15	1 (1.6)	0	1.00

Data are presented as mean ± SD or number (percentage).

CVP, cardiovascular profile.

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SUPPLEMENTAL TABLE 3

Baseline maternal examination data in patients with and without fetal heart failure at 28–33 weeks of gestation (n = 67)

Variables	CVP score ≥8 (n=61)	CVP score ≤7 (n=6)	*P* value
AST, IU/L	10.5 ± 5.5	8.5 ± 3.4	.39
ALT, IU/L	17.4 ± 5.3	15.2 ± 3.2	.32
LDH, IU/L	176.8 ± 37.4	162.2 ± 20.5	.36
Total bilirubin, mg/dL	0.5 ± 0.2	0.4 ± 0.1	.69
BUN, mg/dL	8.2 ± 2.2	7.0 ± 0.6	.20
Creatinine, mg/dL	0.5 ± 0.1	0.4 ± 0.1	.08
White blood cells, 1000/μL	8.2 ± 2.1	7.2 ± 2.8	.28
Hematocrit, %	33.4 ± 2.7	32.1 ± 2.9	.19
Platelets, 10,000/μL	224.5 ± 61.0	228.0 ± 41.5	.89

Data are presented as mean ± SD or number (percentage).

ALT, alanine aminotransferase; *AST*, aspartate aminotransferase; *BUN*, blood urea nitrogen; *CVP*, cardiovascular profile; *LDH*, lactate dehydrogenase.

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SUPPLEMENTAL TABLE 4

Trends of maternal cytokine and hormone concentrations during pregnancy and postpartum in controls

Variables	Gestational age at 28–33 weeks (n = 45)	Gestational age at 34–39 weeks (n = 50)	Postpartum 1 week (n = 45)
Higher in pregnancy[a]			
TNF-α, pg/mL	33.0 ± 15.1	38.8 ± 20.4	28.2 ± 4.2
sFASL, pg/mL	367.7 ± 141.5	423.8 ± 221.6	282.7 ± 59.5
TGF-α, pg/mL	86.1 ± 28.9	93.5 ± 37.3	75.6 ± 14.1
uPA, pg/mL	522.4 ± 182.2	543.7 ± 252.5	346.1 ± 127.9
PDGF-AB/BB, pg/mL	4858.7 ± 1244.7	4985.2 ± 1740.8	3642.9 ± 1545.5
hCG, mIU/mL	25223.2 ± 18365.9	26215.9 ± 16352.4	552.3 ± 1130.3
AFP, ng/mL	259.8 ± 95.6	280.8 ± 132.4	104.1 ± 63.8
sEGFR, pg/mL	22480.1 ± 4298.1	25575.6 ± 5212.2	20001.0 ± 4532.8
Follistatin, pg/mL	2902.0 ± 1222.1	27,29.1 ± 1435.5	1169.2 ± 499.0
G-CSF, pg/mL	137.5 ± 29.7	135.9 ± 27.2	121.6 ± 20.9
sHER2/neu, pg/mL	3979.0 ± 755.5	4850.9 ± 1026.6	3577.6 ± 786.5
Leptin, pg/mL	7118.6 ± 4492.9	8095.5 ± 5582.1	3943.8 ± 3266.7
PECAM-1, pg/mL	3821.8 ± 435.2	3841.3 ± 522.1	3528.5 ± 572.1
sVEGFR-1, pg/mL	1540.2 ± 563.3	47,46.1 ± 4240.1	776.4 ± 220.0
sVEGFR-2, pg/mL	2618.0 ± 576.3	2400.2 ± 567.3	2023.7 ± 532.6
Angiopoietin-2, pg/mL	3868.2 ± 2636.9	2767.0 ± 1934.9	1289.5 ± 408.2
Endoglin, pg/mL	2613.7 ± 2019.3	4433.9 ± 3149.8	2716.2 ± 1349.8
IL-18, pg/mL	126.9 ± 51.2	140.1 ± 69.7	96.4 ± 31.0
PLGF, pg/mL	328.3 ± 266.3	195.0 ± 175.8	62.3 ± 12.2
Higher postpartum[b]			
sCD40L, pg/mL	445.8 ± 86.3	466.9 ± 133.2	546.0 ± 103.2
EGF, pg/mL	55.9 ± 18.2	64.1 ± 32.5	74.1 ± 37.8
HB-EGF, pg/mL	162.9 ± 46.0	159.0 ± 58.6	189.4 ± 26.8
Osteopontin, pg/mL	55671 ± 41251.1	64568 ± 27201.5	115723 ± 50013.0
Prolactin, pg/mL	175612 ± 83771	207807 ± 94622	250230 ± 119654
VEGF-A, pg/mL	436.7 ± 70.1	415.7 ± 78.1	506.4 ± 105.4

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(continued)

SUPPLEMENTAL TABLE 4

Trends of maternal cytokine and hormone concentrations during pregnancy and postpartum in controls *(continued)*

Variables	Gestational age at 28–33 weeks (n = 45)	Gestational age at 34–39 weeks (n = 50)	Postpartum 1 week (n = 45)
No significant difference[c]			
IL-6, pg/mL	59.7 ± 24.2	69.1 ± 34.8	56.2 ± 9.5
VEGF-D, pg/mL	988.7 ± 112.8	995.1 ± 152.5	958.4 ± 73.9
FGF-basic, pg/mL	273.4 ± 34.9	272.0 ± 35.5	257.8 ± 25.3
HGF, pg/mL	1516.3 ± 624.5	1679.3 ± 1379.7	1543.5 ± 746.2
sIL-6Ra, pg/mL	12949.0 ± 3338.9	12753.5 ± 3301.6	12922.7 ± 3770.6
SCF, pg/mL	192.9 ± 27.8	196.0 ± 30.4	184.3 ± 28.8
sTie-2, pg/mL	5159.1 ± 1468.9	4819.4 ± 1428.9	4969.0 ± 1617.7
IGFBP-1, pg/mL	61417.6 ± 20795.7	62155.8 ± 32689.8	74577.6 ± 27258.1
IL-8, pg/mL	24.1 ± 5.1	26.1 ± 7.5	62.2 ± 184.3
PAI-1, pg/mL	68704.7 ± 28927.5	77313.6 ± 33828.5	70955.2 ± 14942.7
VEGF-C, pg/mL	1469.2 ± 209.2	1377.4 ± 245.0	1416.9 ± 245.0

Tukey-Kramer honest significant difference test was used ($P < .05$ indicates a significant difference).

AFP, alpha-fetoprotein; *EGF*, epidermal growth factor; *FGF*, fibroblast growth factor; *G-CSF*, granulocyte-colony stimulating factor; *HB-EGF*, heparin-binding epidermal growth factor-like growth factor; *hCG*, human chorionic gonadotropin; *HGF*, hepatocyte growth factor; *IGFBP*, insulin-like growth factor binding protein; *IL*, interleukin; *PAI*, plasminogen activator inhibitor; *PDGF*, platelet-derived growth factor; *PECAM*, platelet and endothelial cell adhesion molecule; *PLGF*, placental growth factor; *sCD40L*, soluble CD40 ligand; *SCF*, stem cell factor; *sEGFR*, soluble epidermal growth factor receptor; *sFASL*, soluble Fas ligand; *sHER*, soluble human epidermal growth factor receptor; *sVEGFR*, soluble vascular endothelial growth factor receptor; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor; *uPA*, urokinase-type plasminogen activator; *VEGF*, vascular endothelial growth factor.

[a] Serum concentrations were significantly reduced after delivery compared with those during pregnancy; [b] Serum concentrations were significantly increased after delivery compared with those during pregnancy; [c] Serum concentrations were not significantly different during pregnancy and after delivery.

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