Serum Inflammatory Markers and Preeclampsia in Type 1 Diabetes

A prospective study

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OBJECTIVE—Inflammation and endothelial dysfunction have been associated with the immunobiology of preeclampsia (PE), a significant cause of adverse pregnancy outcomes. The prevalence of PE is elevated several fold in the presence of maternal type 1 diabetes mellitus (T1DM). Although cross-sectional studies of pregnancies among women without diabetes have shown altered inflammatory markers in the presence of PE, longitudinal studies of diabetic women are lacking. In maternal serum samples, we examined the temporal associations of markers of inflammation with the subsequent development of PE in women with T1DM.

RESEARCH DESIGN AND METHODS—We conducted longitudinal analyses of serum C-reactive protein (CRP), adhesion molecules, and cytokines during the first (mean ± SD, 12.2 ± 1.9 weeks), second (21.6 ± 1.5 weeks), and third (31.5 ± 1.7 weeks) trimesters of pregnancy (visits 1–3, respectively). All study visits took place before the onset of PE. Covariates were BMI, HbA_{1c}, age of onset, duration of diabetes, and mean arterial pressure.

RESULTS—In women with T1DM who developed PE versus those who remained normotensive, CRP tended to be higher at visits 1 ($P = 0.07$) and 2 ($P = 0.06$) and was significantly higher at visit 3 ($P < 0.05$); soluble E-selectin and interferon-γ–inducible protein-10 (IP-10) were significantly higher at visit 3; interleukin-1 receptor antagonist (IL-1ra) and eotaxin were higher and lower, respectively, at visit 2 (all $P < 0.05$). These conclusions persisted following adjustment for covariates.

CONCLUSIONS—In pregnant women with T1DM, elevated CRP, soluble E-selectin, IL-1ra, and IP-10 and lower eotaxin were associated with subsequent PE. The role of inflammatory factors as markers and potential mechanisms of the high prevalence of PE in T1DM merits further investigation.

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Preeclampsia (PE), characterized by the new onset of hypertension and proteinuria after midgestation, disproportionately affects pregnancies in women with type 1 diabetes mellitus (T1DM) (1). In general, immune aberrations, mainly originating in the placenta and leading to maternal inflammation and endothelial dysfunction, have been associated with PE (2). Existing studies of maternal circulating inflammatory molecules, especially C-reactive protein (CRP), adhesion molecules, cytokines, and chemokines, in pregnancies with and without PE are mostly cross-sectional and do not address pregnancy in diabetic women. In the absence of diabetes, prospective data suggest that markers of inflammation and endothelial dysfunction, especially CRP and adhesion molecules, may serve as potential markers for increased risk of PE (3,4). Further prospective clinical investigations are needed to define the role of these inflammatory factors as markers or mechanisms for PE in the context of T1DM.

Cross-sectional studies of pregnancies affected by PE in nondiabetic women have shown altered maternal levels of CRP, adhesion molecules, and cytokines: CRP levels (5,6) and specific cytokines and chemokines, such as interleukin (IL)-1, −6, and −8; IL-1 receptor antagonist (IL-1ra); interferon (IFN)-γ–inducible protein-10 (IP-10); and monocyte chemoattractant protein-1 (MCP-1), were significantly elevated in women with PE versus healthy pregnant and nonpregnant controls (6–9), whereas IL-1β, IL-4, IL-12, and IFN-γ were not different (6,7). Levels of maternal adhesion molecules, such as soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1), also have been shown to be significantly elevated in women with PE versus healthy pregnant and nonpregnant controls (6). However, these case-control studies do not address the temporal associations of CRP, cytokines, and chemokines with the development of PE. Longitudinal studies of nondiabetic pregnant women who subsequently developed PE show

significant elevations in CRP before the onset of PE (3), but conflicting results were obtained concerning adhesion molecules (10,11) and cytokines (12). One longitudinal study by Clausen et al. (13) of pregnant women with T1DM showed elevated plasma VCAM-1 and ICAM-1, but not E-selectin or vonWillebrand Factor, at 11 weeks' gestation in those who subsequently developed PE versus those who did not. No comprehensive longitudinal data have been reported in pregnancies with or without T1DM to define the levels of key inflammatory molecules (CRP, adhesion molecules, cytokines, and chemokines) in a single cohort. Such a study might provide better insight into the inflammatory milieu preceding PE.

In our prospective study of pregnancies in women with T1DM, we previously reported altered circulating antiangiogenic factors (14), antioxidant carotenoids (15), and cholesterol-rich lipoprotein particles (16) early in pregnancy in women who subsequently developed PE. In the same prospective cohort, we have now evaluated the roles of maternal CRP, adhesion molecules, and cytokines in the subsequent development of PE in women with T1DM. Our specific goal was to define the temporal course of CRP, adhesion molecules (ICAM-1, VCAM-1, E-selectin, P-selectin) and multiple markers related to inflammation and angiogenesis, including cytokines and chemokines, at three gestational "visits," all before onset of PE. We examined differences in these variables at each individual visit (visits 1, 2, and 3) and longitudinally (visits 1–3) between women with T1DM who did versus did not subsequently develop PE. We included a small group of nondiabetic, normotensive pregnant women to define normal values and to enable a secondary comparison between "healthy" diabetic and nondiabetic women. This study does not address PE in the absence of T1DM.

RESEARCH DESIGN AND METHODS

Study design and participants

The participants, study design, and enrollment criteria have been described previously (14,15). Briefly, the study was conducted according to the principles of the Declaration of Helsinki and was approved by the institutional review boards of all participating institutions in Australia, Norway, and the U.S. All participants provided written informed consent. Over a 4-year period, 151 pregnant women with established T1DM and 24 nondiabetic pregnant women were recruited during their first trimester (~12 weeks) then followed throughout the pregnancy. Clinical data and blood and urine specimens were collected at 12.2 ± 1.9, 21.6 ± 1.5, and 31.5 ± 1.7 weeks of gestation (mean ± SD; no overlap). These three visits corresponded with late first, midsecond, and early third trimesters and, in cases of PE, preceded the onset of PE. PE was defined as new-onset hypertension (>140/90 mmHg) accompanied by proteinuria (>300 mg/24 h) after 20 weeks' gestation in a previously normotensive woman (15). The current study uses a subset of our larger cohort (14): of the original 26 women with T1DM and PE, 23 were available for the current study (3 lost as a result of sample attrition). Women without PE but who developed pregnancy-induced hypertension were excluded from the analysis. Of the original 95 normotensive diabetic women, 26 were selected matching for age, duration of diabetes, HbA_{1c}, and parity; of these 26 women, 23 were studied (3 lost as a result of sample attrition). Twenty nondiabetic women (DM-) were included for reference values.

Laboratory measures

Serum CRP was measured by nephelometry as previously described (17). Serum adhesion molecules (soluble E-selectin [sE-Selectin], soluble P-selectin [sP-Selectin], sICAM-1, and sVCAM-1) were measured using the Quantikine human immunoassay kits (R&D Systems, Minneapolis, MN) based on the manufacturer's protocols. Inflammatory factors in serum were measured using the Biometric multiplex assay (Bio-Rad Inc., Hercules, CA) as described previously (18). Of 22 factors related to inflammation, nine were detectable in most (≥95%) samples at two or more visits: IL-1ra, IL-8, IL-12, MCP-1, IP-10, macrophage inflammatory protein (MIP) 1-α, MIP-1β, eotaxin, and regulated upon activation normal T cell expressed and secreted (RANTES). The remaining factors were detectable in <50% of samples at one or more visits: granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-13, IL-15, IL-17, and tumor necrosis factor (TNF)-α. The intra- and interassay coefficients of variation for each of these variables was ≤10%. All assays were performed by operators blinded to sample identity.

Statistical analysis

Our primary analysis considered differences between women with T1DM who subsequently developed PE (DM PE+) versus those who remained normotensive (DM PE-). Between-group tests for CRP, adhesion molecules (sE-selectin, sP-selectin, sICAM-1, sVCAM-1) and each selected cytokine (IL-1ra, IL-8, IL-12, MCP-1, IP-10, MIP-1α, MIP-1β, eotaxin, and RANTES) were performed at each visit using general linear models with and without the following covariates: BMI, HbA_{1c}, age at onset of diabetes, duration of diabetes, and mean arterial pressure. Covariates were selected according to baseline differences, known associations with PE, or both. Nonparametric Mann-Whitney tests were used as appropriate. Between-group tests across all visits were performed using generalized estimating equations (GEEs), which also were performed with and without covariates. Both log-transformed and untransformed values for CRP and cytokines were considered: the conclusions were unchanged by transformation. Results were expressed for illustrative purposes as means ± SDs. All tests were two-tailed, with $P < 0.05$ considered as significant. No adjustments were made for multiple hypothesis testing. A secondary comparison between DM PE- and DM- pregnancies, performed to discern changes attributable to diabetes, was exploratory. Statistical analyses used SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, IL).

RESULTS—Baseline (visit 1) characteristics, as shown in Table 1, showed no significant differences in age, percentage of alcohol users and smokers, pregnancy outcomes (gravida, parity, abortus), age of onset and duration of T1DM, systolic and diastolic blood pressure, microalbuminuria, and gestational age at visit 1 between the DM PE+ and DM PE- groups. However, BMI and HbA_{1c} were significantly higher in the DM PE+ compared with the DM PE- group. Between DM- and DM PE- women, no significant differences were noted at visit 1 except HbA_{1c}, which was significantly higher in the diabetic versus nondiabetic pregnant women.

Data for our main outcome measures are shown in Table 2 and Supplementary Fig. 1. Maternal serum CRP tended to be higher at visit 1 ($P = 0.07$) and visit 2 ($P = 0.06$) and was significantly higher at visit 3 in the DM PE+ versus the DM PE- group ($P < 0.05$). Interestingly, at visit 3 (still

Table 1—*Baseline characteristics of 46 women with type 1 diabetes and 20 nondiabetic controls*

Characteristics	DM PE+ (n = 23)	P value*	DM PE− (n = 23)	P value†	DM− (n = 20)
Age (years)	28.4 ± 5.5	0.31	30.0 ± 4	0.17	32 ± 5
BMI (kg/m^2)	28.0 ± 6.0	**0.04**	24.7 ± 4.2	0.41	23.6 ± 4.0
Alcohol use (%)‡					
None	18	0.39	25	0.55	11
Stopped during pregnancy	68	—	58	—	68
Smoker (%)‡					
No	91	0.69	88	0.55	100
Quit because of pregnancy	5	—	4	—	0
First pregnancy (%)	74	0.74	70	0.27	58
Gravida (n)	1.3 ± 0.7	0.99	1.3 ± 0.7	0.24	1.7 ± 1.0
Para (n)	0.2 ± 0.5	0.91	0.2 ± 0.5	0.27	0.5 ± 1.0
Abortus (n)	0.1 ± 0.4	0.91	0.1 ± 0.3	0.85	0.2 ± 0.4
Age at onset of T1DM (years)	11.5 ± 5.5	0.05	15.4 ± 7.5	—	—
Duration of T1DM (years)	16.8 ± 6.8	0.27	14.5 ± 7.1	—	—
HbA_{1c} (%)	7.3 ± 1.2	**0.04**	6.7 ± 1.0	**<0.001**	5.4 ± 0.3
Blood pressure (mmHg)					
Systolic	113 ± 12	0.25	109 ± 10	0.35	112 ± 9
Diastolic	67 ± 9	0.27	64 ± 8	0.24	67 ± 8
Microalbumin (mg/dL)	1.0 ± 1.8	0.12	0.4 ± 0.2	0.58	0.5 ± 0.2
Gestational age (weeks)					
Visit 1	12.3 ± 2.1	1.00	12.3 ± 1.7	0.84	12.4 ± 1.6
Visit 2	22.1 ± 1.6	0.44	21.5 ± 1.3	0.96	21.4 ± 1.3
Visit 3	31.7 ± 1.7	0.64	31.3 ± 1.5	0.80	31.2 ± 1.1

Values are presented as means ± SD. Measurements refer to visit 1 unless otherwise indicated. P values <0.05 (statistically significant) are bolded. *P value, DM PE+ vs. DM PE−. †P value, DM PE− vs. DM−. ‡P values refer to combined percentage (i.e., "none" and "stopped during pregnancy" or "no" and "quit because of pregnancy").

before onset of PE), CRP levels in the DM PE+ group were double those in the DM PE- group. GEE analyses revealed that, overall, CRP levels were higher in the DM PE+ group than in the DM PE- group throughout pregnancy (visits 1–3).

While maternal sE-selectin, sP-selectin, sICAM-1, and sVCAM-1 (except visit 1) were generally higher at all visits in the DM PE+ versus the DM PE- groups, only sE-selectin reached significance and only at visit 3 ($P < 0.05$). GEE analyses of E-selectin revealed that, overall, levels were higher in the DM PE+ versus the DM PE- group throughout pregnancy (visits 1–3), and in GEE analyses of sICAM-1, a similar trend was observed (visits 1–3; $P = 0.06$).

Among the serum cytokines, IL-1ra was significantly higher only at visit 2 and IP-10 was significantly higher only at visit 3, whereas eotaxin was significantly lower only at visit 2 ($P < 0.05$) in the DM PE+ versus the DM PE- group. No significant differences at any visit were found for IL-8, IL-12, MCP-1, MIP-α, MIP-β, and RANTES. However, GEE analyses revealed borderline overall elevation of IL-8 in the DM PE+ versus the DM PE- group throughout pregnancy (visits 1–3; $P = 0.05$). Levels of 13 cytokines detected in <50% samples (GM-CSF, IFN-α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-13, IL-15, IL-17, and TNF-α) in the two diabetic groups and nondiabetic controls are shown in Supplementary Fig. 1 (graphs XV–XXVII).

All of the significant differences between the DM PE+ and DM PE- groups, as described above, persisted after covariate adjustments. Secondary analyses showed no significant differences in the serum markers at any visit in the DM- versus the DM PE- group, except that MCP-1 was lower in the DM- group at visit 3 ($P < 0.05$). In addition, GEE analyses revealed that, overall, MCP-1 and RANTES levels were lower in the DM- versus the DM PE- group throughout pregnancy (visits 1–3), whereas MIP-1α exhibited a similar trend ($P = 0.07$).

CONCLUSIONS—Our prospective study reveals higher serum CRP, sE-selectin, and specific cytokines (IL-1ra and IP-10) and lower eotaxin in pregnant women with T1DM who subsequently developed PE compared with those who remained normotensive. In general, serum CRP, adhesion molecules, and cytokines were higher at most visits in women with T1DM who later developed PE than in those who remained normotensive. This suggests that exacerbated maternal inflammatory responses confer susceptibility to PE. Because these significant findings persisted following adjustments for covariates, our results show independent temporal associations of these selected inflammatory mediators with PE in women with pregestational T1DM.

CRP, an acute-phase protein produced by the liver in response to proinflammatory cytokines, has been used routinely as a biomarker to monitor progression of disease and response to treatment in patients with inflammatory diseases (19). Multiple epidemiological studies provide strong evidence that CRP can serve as an independent predictor of future vascular events, including risks of hypertension, in nonpregnant populations (20,21). Maternal CRP has been positively correlated with PE in several cross-sectional and longitudinal studies of pregnancies in nondiabetic women (3,5–7,22,23), but no such data have been reported previously in pregnancies complicated by T1DM. Our longitudinal study shows serum CRP to be elevated during the first and second trimesters and significantly elevated during the third trimester in women with T1DM who subsequently developed PE versus those who did not. Associations between CRP and PE largely have been explained by maternal obesity and BMI in case-control and longitudinal studies of pregnancies in nondiabetic women (3,22,23). These studies also report greater risks of PE associated with maternal CRP levels of approximately ≥5.0 mg/L, corresponding to the observed levels of serum CRP in our patients with PE. In addition, following adjustment for maternal BMI, our data show that CRP remains significantly associated with PE. This independent association, even in the presence of elevated maternal BMI, might be explained by the role of pregestational T1DM in producing exaggerated maternal inflammatory responses in pregnancies in diabetic compared with nondiabetic women.

In our study, GEE analyses revealed elevated CRP throughout pregnancy in

Table 2—*Serum markers of inflammation in women with T1DM with and without subsequent PE and in nondiabetic pregnant controls*

Variables by visit	DM PE+ (n = 23)	P value[a]	P value (GEE)[b]	DM PE− (n = 23)	P value[c]	P (GEE)[d]	DM− (n = 20)
CRP (mg/L)							
Visit 1	7.2 ± 7.03	0.07		4.8 ± 7.17	0.22		6.6 ± 10.30
Visit 2	9.9 ± 8.90	0.06		5.7 ± 4.47	0.22		6.3 ± 10.58
Visit 3	11.6 ± 12.26	**0.01**		5.0 ± 3.07	0.42		6.1 ± 8.76
Visits 1–3			**0.01**			0.62	
sE-selectin (ng/mL)							
Visit 1	43.8 ± 14.10	0.34		37.6 ± 15.20	0.19		30.0 ± 10.20
Visit 2	37.3 ± 14.20	0.19		30.2 ± 11.40	0.46		27.5 ± 11.50
Visit 3	41.1 ± 16.50	**0.03**		30.0 ± 12.10	0.98		29.1 ± 10.90
Visits 1–3			**0.04**			0.28	
sP-selectin (ng/mL)							
Visit 1	121 ± 78	0.94		112 ± 91	0.39		80 ± 37
Visit 2	116 ± 57	0.61		100 ± 37	0.62		102 ± 55
Visit 3	126 ± 85	0.47		103 ± 40	0.96		97 ± 50
Visits 1–3			0.25			0.33	
sICAM-1 (ng/mL)							
Visit 1	210 ± 39	0.13		185 ± 47	0.99		186 ± 31
Visit 2	232 ± 66	0.40		204 ± 65	0.63		224 ± 69
Visit 3	238 ± 110	0.13		193 ± 46	1.00		192 ± 24
Visits 1–3			0.06			0.57	
sVCAM-1 (ng/mL)							
Visit 1	746 ± 142	0.72		785 ± 187	0.95		769 ± 119
Visit 2	669 ± 147	1.00		673 ± 155	0.96		688 ± 185
Visit 3	639 ± 106	1.00		637 ± 137	0.85		661 ± 148
Visits 1–3			0.72			0.85	
IL-1ra (pg/mL)							
Visit 1	261 ± 165	0.53		212 ± 153	0.97		201 ± 105
Visit 2	278 ± 126	**0.01**		186 ± 124	0.44		245 ± 184
Visit 3	320 ± 148	0.05		281 ± 264	0.32		195 ± 89
Visits 1–3			0.13			0.68	
IL-12 (pg/mL)							
Visit 1	134 ± 85	0.66		117 ± 51	1.00		119 ± 32
Visit 2	104 ± 46	0.56		91 ± 44	0.89		85 ± 28
Visit 3	100 ± 60	0.34		81 ± 38	0.05		64 ± 24
Visits 1–3			0.26			0.42	
IL-8 (pg/mL)							
Visit 1	28.1 ± 58.70	0.25		10.3 ± 5.70	0.97		12.9 ± 6.50
Visit 2	25.1 ± 47.20	0.33		11.1 ± 6.50	0.88		16.0 ± 21.80
Visit 3	15.8 ± 11.70	0.38		11.1 ± 6.00	0.90		12.7 ± 15.10
Visits 1–3			0.05			0.31	
MCP-1 (pg/mL)							
Visit 1	554 ± 228	0.53		646 ± 367	0.30		514 ± 168
Visit 2	519 ± 225	0.85		554 ± 200	0.78		508 ± 185
Visit 3	555 ± 240	0.71		507 ± 181	**0.03**		387 ± 168
Visits 1–3			0.65			**0.03**	
IP-10 (pg/mL)							
Visit 1	18.3 ± 11.60	0.99		18.3 ± 12.80	0.91		20.0 ± 14.10
Visit 2	17.4 ± 9.30	0.87		18.8 ± 7.70	0.38		15.2 ± 7.30
Visit 3	23.5 ± 14.70	**0.02**		14.7 ± 8.20	0.99		14.2 ± 7.20
Visits 1–3			0.27			0.74	
MIP-1α (pg/mL)							
Visit 1	233 ± 103	0.62		215 ± 15	0.96		209 ± 17
Visit 2	234 ± 99	0.64		216 ± 17	0.93		208 ± 21
Visit 3	227 ± 124	0.64		207 ± 17	0.96		200 ± 12
Visits 1–3			0.4			0.07	

Continued on p. 2058

Table 2—*Continued*

Variables by visit	DM PE+ (n = 23)	P value[a]	P value (GEE)[b]	DM PE− (n = 23)	P value[c]	P (GEE)[d]	DM− (n = 20)
MIP-1β (pg/mL)							
Visit 1	204 ± 39	0.57		193 ± 34	0.24		212 ± 32
Visit 2	205 ± 38	0.99		203 ± 23	0.35		218 ± 36
Visit 3	195 ± 33	0.99		193 ± 32	0.99		195 ± 24
Visits 1–3			0.57			0.13	
Eotaxin (pg/mL)							
Visit 1	98.9 ± 29.7	0.57		104 ± 29.3	0.52		94.7 ± 15.7
Visit 2	81.5 ± 21.4	**0.02**		96.3 ± 19.5	0.87		92.9 ± 21.6
Visit 3	72.5 ± 22.8	0.41		78.2 ± 24.2	0.27		67.9 ± 13.3
Visits 1–3			0.19			0.15	
RANTES (pg/mL)							
Visit 1	6,540 ± 4,115	0.14		8,477 ± 4,455	0.14		5,913 ± 3,739
Visit 2	8,143 ± 4,438	0.63		8,751 ± 3,806	0.27		6,634 ± 3,992
Visit 3	5,907 ± 4,387	0.75		6,329 ± 4,608	0.27		4,403 ± 1,516
Visits 1–3			0.35			**0.02**	

Values are means ± SD for illustrative purposes. P values are reported from parametric, log-transformed, or nonparametric analyses as appropriate (see RESEARCH DESIGN AND METHODS). P values <0.05 (statistically significant) are bolded. [a]P value, DM PE+ vs. DM PE− at each visit (1–3). [b]P value, GEE of DM PE+ vs. DM PE− across visits 1–3. [c]P value, DM PE− vs. DM− at each visit (1–3). [d]P value, GEE of DM PE− vs. DM− across visits 1–3.

diabetic women who later developed PE versus those who remained normotensive. Interestingly, we observed minimal changes in CRP throughout pregnancy (visits 1–3) in normotensive women with T1DM. These findings are supported by previously reported data from non-PE pregnancies in women with T1DM, in which maternal CRP showed no significant overall changes with advancing pregnancy, except when stratified by glycemic control status (24). Thus, our longitudinal study shows increasing CRP with advancing pregnancy to be an indicator of enhanced maternal systemic inflammation and of risk for PE in T1DM.

Endothelial dysfunction, characterized by increased expression of adhesion molecules (sICAM-1, sVCAM-1) and members of the selectin (E and P) family, has been shown to promote endothelial adhesion and accumulation of inflammatory cells, thus initiating the development of vascular disease (25,26). Maternal levels of adhesion molecules have been associated with PE in case-control and longitudinal studies of pregnancies in nondiabetic women. However, results are conflicting: the data show elevated (6,10,11), lower (11), and/or similar (11) levels of one or more adhesion molecules to be associated with subsequent development of PE. In the context of pregnancy in women with T1DM, a single longitudinal study by Claussen et al.(13) showed elevated plasma levels of VCAM-1 and ICAM-1, but not E-selectin, at 11 weeks' gestation in women who subsequently developed PE versus those who did not. Our longitudinal study assessed levels of adhesion molecules at three "visits" before the onset of PE and revealed significantly higher sE-selectin at visit 3 only (~32 weeks) in women who later developed PE versus those who did not. We did not observe any significant differences in levels of sP-selectin, sICAM-1, or sVCAM-1 in association with PE. Differences between our study and that of Claussen et al. might be explained by differences in group size, timing of sample collections, and overall glycemic control. While adhesion molecules have been strongly correlated with endothelial dysfunction in nonpregnant cohorts with T1DM (27,28), their roles in the development of hypertensive complications, including PE, in pregnancies in women with T1DM remain to be defined in detail.

In the current study, GEE analyses showed no significant differences in levels of adhesion molecules throughout pregnancy in women with T1DM who later developed PE versus those who did not. However, while the change in sE-selectin was minimal throughout pregnancy in the DM PE+ group, the DM PE- group showed a decrease in sE-selectin with advancing pregnancy. In a previously reported longitudinal study in non-PE pregnancies in women with T1DM, no significant changes were noted in E-selectin and ICAM-1, whereas VCAM-1 increased significantly between 12 and 36 weeks of gestation (29). The discrepancies between this study and ours might be explained by maternal characteristics, including overall glycemic control and timing of sample collections.

Cytokines and chemokines are considered to be immunological markers and have been implicated in endothelial dysfunction and subsequent vascular abnormalities (30). While an increase in cytokines during pregnancy reflects successful implantation and placentation, an imbalance between cell-mediated immunity type 1 helper T cells (TH1) (e.g., IL-12) and allergic immunity type 2 helper T cells (TH2) (e.g., IL-4)–type cytokines has been implicated in PE (31,32). Several cytokines and chemokines have been reported to be altered in PE in cross-sectional and longitudinal studies of pregnancies in nondiabetic women (6–9,12). However, no data examining the associations between levels of cytokines and chemokines and the subsequent onset of PE have been reported in pregnant women with T1DM. In our prospective study, we observed significantly elevated IL-1ra and IP-10 at the midsecond and early third trimesters, respectively, before the onset of PE in the DM PE+ versus the DM PE- group. These specific cytokines have been correlated with

β-cell function and progression of T1DM. As a natural antagonist to IL-1β, which is thought to contribute to β-cell destruction, IL-1ra has been identified as an anti-inflammatory cytokine (33) and has been shown to be elevated in nondiabetic women with PE (6,34,35). The increased levels of IL-1ra indirectly reflect increased activities of IL-1α and -β; both have a very short half-life and are often difficult to detect (34,35). On the other hand, IP-10 is considered a proinflammatory cytokine contributing to the progression of T1DM (36); it has been shown to be elevated in studies of PE in pregnancies of nondiabetic women (6,35). We also observed significantly lower eotaxin at the midsecond trimester in women with T1DM who subsequently developed PE compared with those who remained normotensive. Eotaxin, a less commonly measured proinflammatory cytokine in the context of PE, previously has been shown to be similar between PE and non-PE cases (9) but has not been reported in other studies of PE in pregnancies of nondiabetic women (6–8,12). Thus, our observation of generally lower eotaxin at all visits in women with T1DM who later developed PE, when compared with those who did not, suggests a possible role for eotaxin in PE and warrants further investigation.

Although our study provides novel evidence of the temporal associations of IL-1ra, IP-10, and eotaxin with subsequent PE in pregnancies of women with T1DM, we were not able to detect many other proinflammatory cytokines associated with PE, such as IL-6 and TNF-α (12), in the majority of our samples. Our results conform to previously reported data from pregnancies in nondiabetic women, in which several cytokines, such as GM-CSF, IFN-γ, or TNF-α, were below detection limits in maternal serum samples at one or more visits (12). Thus, individual and/or synergistic function of cytokines in the development of PE in T1DM needs to be defined further in larger studies defining effects of gestational age or differences in analytical methods.

In our study, GEE analyses showed no significant differences in levels of any cytokines with advancing pregnancy between the DM PE+ and DM PE- groups. We observed a borderline significant decrease in IL-8 with advancing pregnancy in women with T1DM who later developed PE versus those who did not. Our results conform to previously reported data in non-PE pregnancies in women with T1DM, showing no differences in levels of proinflammatory cytokines with advancing pregnancy, even when stratified by glycemic status (24).

Our secondary analyses revealed no significant changes in any of the markers of inflammation, except MCP-1, which was lower in the DM- versus the DM PE- group. These results conform to some previous studies showing no significant differences in inflammatory markers (CRP, IL-6, VCAM-1) between women with T1DM and nondiabetic women throughout pregnancy (24), but do not conform to others showing elevated CRP but not VCAM-1 in subjects with T1DM versus nondiabetic control subjects (37). Thus, inflammatory markers may be differentially modulated with advancing pregnancy in the absence versus presence of T1DM and may be related to metabolic control.

The specific limitations of our study include a small sample size, especially in the diabetic group who later developed PE; the absence of prepregnancy levels of inflammatory markers of interest; and the absence of a nondiabetic PE group. The latter was not included in our study design because it was not feasible (considering time and resources needed given the low PE case yield in nondiabetic women). We did not take multiple hypotheses testing into account, but our significant findings are biologically plausible and consistent with previously reported scientific literature describing nondiabetic pregnancies. Another possible limitation concerns analyses of cytokines, which are highly unstable molecules that are susceptible to variations in time, temperature, and handling procedures (38). Although we ensured optimal techniques at all stages of analysis, variation due to these factors might have contributed to the nondetectable levels of certain cytokines and chemokines. Similar variations in cytokine levels have been reported previously in studies of nondiabetic pregnancies, suggesting significant diversity in cytokine metabolism in physiological and complicated pregnancies (12,39).

In conclusion, our prospective study provides new details of the temporal course of maternal inflammatory markers in pregnancies complicated by T1DM with and without subsequent PE. We found that significantly higher CRP and specific adhesion molecules (sE-selectin) and cytokines (IL-1ra, IP-10) and lower eotaxin were associated with the subsequent development of PE at one or more gestational ages in women with T1DM. In general, levels of CRP, adhesion molecules, and cytokines (except eotaxin) were elevated throughout pregnancy in diabetic women who subsequently developed PE versus those who did not, suggesting that activation of systemic inflammation may be a mediator of PE in the presence of T1DM. Our findings support the conduct of larger studies to confirm the role of inflammation and to define the utility of inflammatory factors in the etiology, early screening, and development of preventive and therapeutic strategies for PE in T1DM.

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