Low maternal serum levels of placenta growth factor as an antecedent of clinical preeclampsia

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Knoxville, Tennessee, Des Moines, Iowa, and Taipei, Taiwan

OBJECTIVE: Maternal serum placenta growth factor levels have been shown to be significantly reduced in women with established preeclampsia. However, the temporal change in serum placenta growth factor levels before the clinical onset of preeclampsia is not known.

STUDY DESIGN: Serum samples were collected from patients at the first prenatal (5-15 weeks’ gestation), second-trimester (16-20 weeks’ gestation), and third-trimester (26-30 weeks’ gestation) visits. Serum placenta growth factor levels were determined and analyzed according to pregnancy outcome.

RESULTS: Maternal placenta growth factor levels during normal gestation increased dramatically from the first to the third trimester. At the same gestational time points, in contrast, significantly lower serum placenta growth factor levels were found in patients in whom mild or severe preeclampsia eventually developed (P < .01). Low maternal serum placenta growth factor levels during early gestation were associated with a significant odds ratio for development of preeclampsia (P < .005).

CONCLUSION: Relatively decreased levels of serum placenta growth factor occur before the onset of clinical preeclampsia, which suggests that placenta growth factor measurement could be used to discriminate those pregnancies predisposed to development of preeclampsia. (Am J Obstet Gynecol 2001;184:1267-72.)

Key words: Placenta growth factor, preeclampsia, pregnancy, trophoblast

Despite extensive advances in obstetric research and clinical implementations, preeclampsia remains one of the leading causes of maternal and fetal morbidity and mortality in developed countries. It would be advantageous to diagnose preeclampsia before its clinical manifestation, because prevention or delay of the onset of this disease would have a significant impact on maternal and perinatal outcomes. However, there is little consensus regarding tests to predict, before the onset of clinical signs or symptoms, which patients will acquire preeclampsia.1

The pathophysiologic development of preeclampsia is multifaceted and likely results from shallow invasion of maternal spiral arteries by endovascular cytotrophoblasts. Consequently, the spiral arteries do not undergo sufficient conversion from low-capacity, high-resistance blood vessels to uteroplacental arteries characterized by high capacity and low resistance to blood flow.2 Because migration of endovascular trophoblasts occurs early in the course of human pregnancy, the disease process of preeclampsia most likely is initiated long before symptoms become clinically evident.

One consequence of shallow migration of the endovascular trophoblast is thought to result in local placental bed hypoxia in preeclampsia. Hypoxia induces significant morphologic changes in cultured trophoblasts,3 alters adhesion molecule expression,4 and reduces differentiation of cytotrophoblasts in vitro.5 These changes are in general agreement with in vivo observations of preeclamptic placentas.6,7 Studies have recently begun to focus on the involvement of angiogenic growth factors in the pathophysiology of the disease, because these factors can induce activation of endothelial cells, a well-recognized hallmark of preeclampsia.8 Among the angiogenic growth factors studied have been vascular endothelial growth factor (VEGF) and placenta growth factor (PIGF). PIGF is a secreted dimeric glycoprotein that shares significant sequence homology with VEGF, a potent angiogenic growth factor. These factors are similar in their actions on endothelial cells.9 Unlike VEGF, however, which is expressed in many organs, PIGF is expressed primarily in the placenta10 and specifically by trophoblasts.11,12 In vitro studies have shown that hypoxia upwardly regu-
lates trophoblast expression of VEGF but downwardly regulates PIGF expression,
which suggests that these factors could be used as biochemical markers of placental function during preeclampsia. Unfortunately, the use of maternal serum levels of VEGF as a predictor of preeclampsia has met with conflicting conclusions (see Torry et al9 for discussion), presumably because of the varied expression origins of the protein. Conversely, the relatively restricted expression patterns of PIGF suggest that it may provide a more definitive and specific indication of placental function. Indeed, we14 and subsequently others15 have documented that serum levels of PIGF are uniformly decreased in women with clinically established preeclampsia.

The purpose of this study was to determine the temporal changes in maternal serum PIGF levels among women with development of preeclampsia. We found that women with subsequent development of preeclampsia had significantly lower serum levels of PIGF during all 3 trimesters than did patients in whom preeclampsia never developed. These results support the potential use of maternal serum PIGF levels as a preclinical marker for those women in whom preeclampsia may develop later in pregnancy. Conceivably, the earlier diagnosis of preeclampsia could enable therapeutic interventions to be initiated sooner, with the hope of lessening the morbidity associated with preeclampsia.

**Material and methods**

**Patients and serum samples.** Pregnant nulliparous women were recruited to participate in the study at the first antenatal visit. Blood samples were drawn during routine prenatal visits, and each patient studied had at least 2 blood samples collected within the gestational time points. Gestational age was derived from last menstrual period and was corrected according to transvaginal ultrasonography if needed. Blood samples were drawn into standard serum tubes, allowed to clot, separated by centrifugation, and stored at −70°C until studied. After delivery the patients were separated into three clinical groups according to criteria established by The American College of Obstetricians and Gynecologists16 as previously reported.14 Control subjects (n = 25) were patients who remained normotensive throughout pregnancy and whose infants were not considered to have intrauterine growth restriction. Patients were considered to have mild preeclampsia (n = 10) if they demonstrated an increase of 30 mm Hg systolic or 15 mm Hg diastolic above documented pressures at <20 weeks’ gestation or a blood pressure of ≥140/90 mm Hg at ≥20 weeks’ gestation and also had proteinuria of ≥20.3 g/24 h. Patients with severe preeclampsia (n = 4) had persistent systolic pressure >160 mm Hg or diastolic pressure >110 mm Hg, urinary protein loss >3 g/24 h, generalized edema, serum uric acid level >5 mg/dL, and hyperreflexia. All blood pressures were measured with the patient in a seated position and with the cuff at the level of the heart. A complete blood cell count was obtained for all the patients, and uric acid level, aspartate aminotransferase activity, blood urea nitrogen, creatinine level, and lactate dehydrogenase activity were obtained from the patients with preeclampsia.

Exclusion criteria for the patient groups were multiparity, chronic hypertension, diabetes, multiple gestation, connective tissue disorder, any long-term use of medicine other than prenatal vitamins, and miscarriage before viability. The study protocol was approved by local institutional review boards, and written informed consent was obtained.

**PIGF-capture enzyme-linked immunosorbent assay.** Levels of serum PIGF were assessed in duplicate with a sensitive PIGF-specific antigen-capture enzyme-linked immunosorbent assay according to the manufacturer’s directions (R&D Systems, Minneapolis, Minn). The enzyme-linked immunosorbent assay recognizes both natural and recombinant human PIGF and exhibits no detectable cross-reactivity with other cytokines or growth factors, other than a 3% cross-reactivity with the recombinant human PIGF-VEGF heterodimer. According to the manufacturer, the enzyme-linked immunosorbent assay has a sensitivity of 5 pg/mL and intra-assay and interassay coefficients of variation of approximately 6% and 11%, respectively, in serum. Most important, antigen detection is not inhibited by soluble flt-1 receptors at ≤2000 pg/mL.

**Statistical analysis.** Significant differences between patient groups for continuous variables were determined by Kruskal-Wallis analysis of variance with the Dunn multiple comparisons post hoc test to determine between-group differences. Proteinuria was analyzed in a 2 × 2 contingency table with the Fisher exact test comparing the normal pregnancy group with each preeclampsia group. Kruskal-Wallis analysis of variance was used to determine statistical significance of differences between PIGF levels in the patient groups at each of the gestational time points, with the Dunn multiple comparisons post hoc test used for between-group comparisons. The main interaction effects between PIGF levels, gestational age, and pregnancy outcome were assessed by 2-way analysis of variance, with gestational age and outcome as independent variables. Receiver operating characteristic curves were examined to determine the optimal cutoff levels for serum PIGF concentration to discriminate the patients with normal pregnancies from those who eventually had clinically diagnosed preeclampsia. These cutoffs were used in 2 × 2 contingency tables to calculate odds ratios for development of preeclampsia, and the Fisher exact test was used to determine the significance of the odds ratios. Results are listed as mean ± SEM unless noted, and statistical significance was attained at P < .05. Statistical analyses were performed with the STATISTICA (StatSoft, Inc, Tulsa, Okla) software package.
Table I. Characteristics of study groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal pregnancy (n = 25)</th>
<th>Mild preeclampsia (n = 10)</th>
<th>Severe preeclampsia (n = 4)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>25.0 ± 4.7</td>
<td>25.0 ± 3.7</td>
<td>25.5 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age at delivery (wk)</td>
<td>39.1 ± 2.0</td>
<td>38.1 ± 1.9</td>
<td>29.8 ± 2.9*</td>
<td>*P = .0018</td>
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<tr>
<td>Birth weight (g)</td>
<td>3042.2 ± 371</td>
<td>3021.0 ± 493.7</td>
<td>1077.7 ± 441.2*</td>
<td>*P = .017</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic, 1st trimester</td>
<td>111 ± 10</td>
<td>112 ± 5.8</td>
<td>122 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic, 1st trimester</td>
<td>68 ± 8.3</td>
<td>66.8 ± 4.1</td>
<td>70 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic, 2nd trimester</td>
<td>108 ± 11</td>
<td>118 ± 10</td>
<td>139 ± 15*</td>
<td>*P = .0016</td>
</tr>
<tr>
<td>Diastolic, 2nd trimester</td>
<td>67 ± 7.6</td>
<td>70 ± 8.2</td>
<td>85 ± 4.1*</td>
<td>*P = .0041</td>
</tr>
<tr>
<td>Systolic, 3rd trimester</td>
<td>116 ± 10</td>
<td>145 ± 9*</td>
<td>192 ± 17*</td>
<td>*P &lt; .0001</td>
</tr>
<tr>
<td>Diastolic, 3rd trimester</td>
<td>70 ± 9.2</td>
<td>91 ± 6.4*</td>
<td>112 ± 5.0*</td>
<td>*P &lt; .0001</td>
</tr>
<tr>
<td>Proteinuria 2+ (No.)</td>
<td>0/25</td>
<td>10/10</td>
<td>4/4</td>
<td>*P &lt; .0001†</td>
</tr>
</tbody>
</table>

All values are listed as mean ± SD. Statistical comparisons between groups were analyzed by Kruskal-Wallis analysis of variance. NS, Not significant.

*Significantly different from normal pregnancy group by Dunn multiple comparisons test.
†Result of Fisher exact test comparison between normal pregnancy group and each preeclampsia group.

Results

Table I summarizes the clinical and demographic data from the normal pregnancy and mild and severe preeclampsia groups. There were no statistically significant differences in maternal age or first-trimester blood pressures between the groups. As anticipated from the grouping criteria, however, systolic and diastolic blood pressures in the second and third trimesters and proteinuria were significantly greater in the preeclampsia groups than in the normal pregnancy group. Gestational age at delivery and birth weight were significantly lower in the severe preeclampsia group than in both the mild preeclampsia and normal pregnancy groups.

Maternal PlGF levels were determined in serum samples from each group of patients collected at 3 gestational time points (5-15, 16-20, and 26-30 weeks’ gestation). Both gestational age and pregnancy outcome were independently associated with serum PlGF levels (P < .0001). In addition, the interaction effect of gestational age and pregnancy outcome significantly (P = .0054) influenced serum PlGF levels. There were statistically significant differences in the mean PlGF level (Fig 1) between the patient groups at the early gestation (P < .0001), mid-second-trimester (P = .0112), and third-trimester (P = .0087) time points. At each time point the relative rank order of patient groups according to mean serum PlGF levels was normal pregnancy greater than mild preeclampsia greater than severe preeclampsia. First-trimester serum PlGF levels were approximately 10-fold lower in the severe preeclampsia group than in the normal pregnancy group (P < .001) and remained approximately 5- to 6-fold lower at both 16 to 20 weeks’ gestation (P < .01) and 26 to 30 weeks’ gestation (P < .05). Mean serum PlGF levels in the cohort with mild preeclampsia showed an intermediate distribution between levels seen in the normal pregnancy group and those seen in the severe preeclampsia group. Levels were significantly lower than in the normal pregnancy group at the early gestational time point (P < .01) but not at 16 to 20 weeks’ gestation or 26 to 30 weeks’ gestation (P > .05). Although levels tended to be higher in the mild preeclampsia group than in the severe preeclampsia group at each gestational time point, the differences did not reach statistical significance. These results are in close agreement with our previously published data, in which levels of PlGF were not strongly correlated with disease severity in women with established preeclampsia.14 Larger clinical studies may be necessary to detect significant differences according to disease severity.

Similar to our previous data,14 PlGF levels in women with normal pregnancy increased dramatically from the first or early second trimester (38.55 ± 7.5 pg/mL) to the late second trimester (175.5 ± 21.64 pg/mL) and continued to increase into the third trimester (753.0 ± 65.7 pg/mL). PlGF levels peaked in the normal pregnancy group at approximately 26 to 30 weeks’ gestation before declining as delivery approached (data not shown). Although serum PlGF levels also increased with gestational age in the mild and severe preeclampsia groups, mean serum PlGF levels in both preeclampsia groups remained lower than the corresponding value of the normal pregnancy group at all gestational time points analyzed.

Further analyses of differences in mean serum PlGF levels between patient groups at each gestational time point suggest that serum PlGF levels may provide a clinically useful indicator for the development of preeclampsia (Table II). Receiver operating characteristic curves were examined to determine the optimal cutoff values of serum PlGF concentration to be used to discriminate normal pregnancy from preeclamptic pregnancy. During the first or early second trimester the optimal cutoff for discrimination of the women with preeclampsia was a serum PlGF level ≤32 pg/mL. This value yielded an odds ratio for development of preeclampsia of 95 (P < .001), with a sensitivity of 0.999 and a specificity of 0.905. On the basis of this sample of women it appears that there is little over-
lap between normal pregnancy and preeclampsia with respect to serum PlGF levels. Similarly, the optimal cutoff during the second trimester for discrimination of the women with preeclampsia was 590 pg/mL, which resulted in an odds ratio of 16 (P = .004), with a sensitivity of 0.667 and a specificity of 0.889. Collectively, these results show that maternal serum PlGF levels are statistically lower in women in whom preeclampsia eventually develops and that the difference becomes detectable before the typical clinical onset of the disease. Validation of these novel findings and determination of their clinical utility await larger clinical studies.

Comment

This is the first study to document the association between preeclampsia and PlGF concentration in a longitudinal fashion. These results confirm our previous cross-sectional data and those of others, which documented that PlGF concentration is significantly lower in patients with preeclampsia. Our study extends those initial findings to show that serum PlGF levels are significantly reduced in patients with preeclampsia relatively early during gestation and clearly before clinical manifestations of preeclampsia are typically evident. Decreased levels of PlGF in women with preeclampsia first became evident during early pregnancy (at ≤15 weeks’ gestation), and the difference continued to increase through the mid third trimester of gestation. These results suggest

<table>
<thead>
<tr>
<th>5-15 wk</th>
<th>16-20 wk</th>
<th>26-30 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odds ratio Value</td>
<td>95</td>
<td>16</td>
</tr>
<tr>
<td>95% Confidence interval</td>
<td>7.6-1180</td>
<td>2.4-106</td>
</tr>
<tr>
<td>Statistical significance</td>
<td>P &lt; .001</td>
<td>P = .004</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.909</td>
<td>0.667</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.905</td>
<td>0.889</td>
</tr>
</tbody>
</table>

A 2 × 2 contingency table (Fisher exact test) was used to calculate the odds ratios and 95% confidence intervals. At each respective time point, receiver operating characteristic curves were used to determine optimal cutoff values for normal versus low PlGF concentrations. Pregnancy outcome was classified as normal pregnancy or preeclampsia.
that PlGF concentration may be used as a preclinical indicator for preeclampsia.

Maternal serum levels of PlGF increased approximately 3-fold from the late first trimester to the second trimester in healthy pregnant women. This increase is consistent with our previous cross-sectional data and probably represents placental requirements to recruit and maintain an adequate placental circulation. This study also confirms our previous findings that serum PI GF levels peak at approximately 26 to 30 weeks’ gestation (approximately 13-fold higher than during the first trimester) and then decrease as term approaches (data not shown). The biologic significance of low PlGF production by preeclamptic trophoblasts is not known; however, presence of the PlGF receptor flt-1 on trophoblasts raises several possibilities. Exogenous PlGF has been shown to induce proliferation but not invasiveness of first-trimester trophoblasts. In term trophoblasts, PlGF has been shown to induce the stress-activated protein kinase pathways Jun-N-terminal kinase and p38, but it does not induce significant extracellularly regulated kinase activity. Activation of the stress-activated protein kinase pathways protects trophoblasts from apoptosis in vitro. The ability of PlGF to regulate apoptosis in trophoblasts may be significant, because the relatively low levels of PlGF produced during the first trimester and near term coincide with those gestational time points at which increased levels of trophoblast apoptosis are normally exhibited. The extremely low serum levels of PlGF noted in preeclampsia also coincide with increased apoptosis noted in preeclamptic placentas.

Trophoblast-derived PlGF may also act in a paracrine manner to influence endothelial cells. PlGF messenger ribonucleic acid expression has been found exclusively in villous trophoblast, whereas protein localization was also noted in the fetal stem vessels, and fetal cord blood levels of PlGF are correlated with the maternal serum levels. Collectively, these data support the hypothesis that trophoblast-derived PlGF would influence both maternal and fetal vessel integrity. This hypothesis is in keeping with the general endothelial cell dysfunction that characterizes preeclampsia. Furthermore, low PlGF production by the preeclamptic placenta may contribute to the relative lack of well-developed uteroplacental vessels in preeclamptic versus normal pregnancies.

The prevalence of preeclampsia and its associated morbidity and mortality have prompted many studies focused on finding a preclinical marker for the disease. Unfortunately, many of the tests advocated have little predictive value, or sufficient power of prospective longitudinal studies for verification is lacking. To be a clinically valuable preclinical marker for preeclampsia, a potential test should be easily reproducible in the first or second trimester, should have a high sensitivity, and should be noninvasive. Monitoring of serum PI GF levels during routine prenatal visits would satisfy these practical concerns. The prominent expression of PlGF by trophoblasts, down-regulation by hypoxia, and effects on trophoblast and endothelial cells make PlGF measurement an attractive candidate for monitoring the development of preeclampsia. Interventions to prevent preeclampsia generally have not been effective, but it is thought that initiation of treatment options at earlier gestational ages may provide a greater therapeutic benefit. Although our evidence supports PlGF concentration as a useful indicator to assist in discriminating those patients at highest risk for development of preeclampsia, determinations of the clinical value of this marker as a screening tool and of its utility in reducing the morbidity and mortality associated with this illness await larger longitudinal prospective treatment studies.

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REFERENCES


