Serum Levels of Vascular Endothelial Growth Factor in Preeclamptic and Normotensive Pregnancy

Alyson Hunter, Mark Aitkenhead, Carolyn Caldwell, Geoffrey McCracken, David Wilson, Neil McClure

Abstract—The purpose of these studies was first to determine if vascular endothelial growth factor (VEGF), a vascular permeability agent, is increased in the serum of women with preclinical and clinical preeclampsia (PE), and second to determine how these levels change after delivery. Twenty preeclamptic and 25 normotensive women at term consented to have blood taken pre- and post-delivery. Ten preeclamptic, 10 gestational hypertensive, and 28 normotensive women had blood collected respectively at 12, 20, and 30 weeks gestation and predelivery. Serum was extracted from all samples, and VEGF concentrations were determined by radioimmunoassay. Predelivery, the median serum VEGF concentration in the preeclamptic group was 51.7 ng/mL, and in the control group the concentration was 13.9 ng/mL ($P<0.0001$). Serum VEGF concentrations fell within 24 hours of delivery in both groups, which resulted in median values of 3.8 ng/mL and 3.2 ng/mL respectively ($P<0.3$). At 12 and 20 weeks, there was no significant difference between the serum VEGF concentrations in the 3 groups ($P<0.3, 0.052$ respectively). At 30 weeks, prior to the onset of clinical PE, the serum VEGF levels in the eventual preeclamptic group were elevated significantly compared with the gestational hypertensive and normotensive groups ($P<0.001$). Predelivery serum VEGF concentrations were significantly elevated in the preeclamptic group and were similar to those in the first study ($P<0.0001$). These findings suggest that VEGF may be important in the pathophysiology of PE and has the potential to act as a preclinical marker for the condition. (Hypertension. 2000;36:965-969.)

Key Words: vascular endothelial growth factor ■ PE ■ endothelium ■ serum ■ radioimmunoassay

Preeclampsia (PE) remains a leading cause of maternal morbidity and mortality, and its cause remains obscure.¹ One plausible hypothesis, supported by a variety of morphological and biochemical observations, is that abnormal placentation results in the release of cytokines and other products that enter the maternal circulation and damage the endothelium.²⁻⁴ One such cytokine is vascular endothelial growth factor (VEGF). The placental production of VEGF may be increased by exaggerated placental hypoxia, and the action of VEGF on endothelium may induce vasospasm and hypertension as well as increased vascular permeability.⁵⁻⁷

VEGF concentrations have been measured in the maternal circulation by different investigators during normal and preeclamptic pregnancies with conflicting results including increases, decreases, and no change.⁸⁻¹¹ The discrepancies may reflect study design as well as the use of a variety of assays that may not always be reliable in pregnancy. Also, different groups may have measured different isoforms of VEGF and different physical states of the cytokine (bound or free). We therefore designed prospective studies to measure VEGF in pregnancy with the use of a carefully validated assay to test the hypothesis that circulating cytokine levels would increase in PE.¹²

The aims of this study were twofold. First, we sought to determine if serum VEGF is elevated at the time of clinical PE and to demonstrate how these values change immediately postdelivery, because PE usually resolves rapidly in the immediate puerperium. Second, we have investigated serum VEGF concentrations prospectively throughout pregnancy to determine if VEGF concentrations alter before the clinical manifestation of the condition.

Methods

These studies were approved by the local Research Ethics Committee, and written consent was obtained from all volunteers. Subjects in both studies were recruited at the Royal Maternity Hospital, Belfast, Northern Ireland. All were nulliparous with singleton pregnancies. None gave a history of hypertension, renal or connective tissue disease, or of antepartum hemorrhage. Preeclampsia (PE) was defined as blood pressure (BP) elevated over 140/90 mm Hg (diastolic BP recorded at Korotkoff 5) on 2 occasions at least 6 hours apart, or an admission BP over 160/110 mm Hg requiring immediate treatment. In addition, the presence of at least +++ proteinuria on Dipstix testing in the absence of urinary tract infection on culture was necessary for the diagnosis. Gestational hypertension (GH) was defined as with PE without proteinuria on any occasion. The remainder of the subjects were classified as normotensive (NT).
Peripheral venous blood was collected from all subjects into glass tubes and allowed to clot at room temperature before being centrifuged at 1200 rpm for 20 minutes at 4°C. The serum was then stored at −20°C in multiple aliquots for analysis.

The radioimmunoassay was performed as described by Anthony12 with recombinant human (rh) VEGF165 (R&D Systems Europe, Ltd) as standard and 125I labeled rh-VEGF (Amersham Pharmacia Biotech UK, Ltd) as tracer. The assay buffer was 0.2% bovine serum albumin in 0.04 mol/L phosphate buffer (pH 7.4) with 625U Trypsylol (Bayer AG) per mL. Aliquots (100 μL) of standard assay buffer with 0.14 mol/L NaCl or unknown serum were incubated with 100 μL polyclonal goat anti-human VEGF antiserum (R&D Systems, Europe Ltd) at an initial dilution of 1:2000. 125I rh-VEGF (100 μL diluted 1:300 in assay buffer) and 3.4 international units (iu) heparin were added to each tube and incubated at 4°C overnight. A polyethylene glycol-assisted double antibody method (donkey anti-goat and carrier normal-goat serum [IDS, UK Ltd]) was used to separate antibody bound 125I rh-VEGF from free 125I rh-VEGF. The radioactivity in the bound fraction was then counted. All VEGF concentrations shown represent the mean value as obtained by 2 separate assays. Two separate standard curves were constructed to allow accurate readings of the samples at the upper and lower ranges of the assays. All of the samples were in the linear range of the standard curves. Intra- and inter-assay coefficients of variation were 5.8% and 10.4% respectively at 4 ng · mL−1. The antibody lacked cross-reactivity for other cytokines as specified in the R&D data sheet. Recovery of VEGF added to nonpregnant serum concentrations across the range of the assay was between 71% and 95% (n = 10). Recovery of VEGF added to pregnant serum concentrations was 0% to 4% (n = 30).

In the first study 20 PE and 25 NT subjects between 37 weeks and 41/3 weeks gestation were recruited, on admission, prior to delivery. Blood samples were taken from the PE subjects once the condition was diagnosed. Control subjects had blood samples collected on admission to the delivery suite. An additional blood sample was collected from both control and PE subjects between 12 and 24 hours after delivery. None of the women in either group developed higher BP or worsening proteinuria after delivery.

In the longitudinal study, 400 nulliparous women consented, at their antenatal booking visit, to have 10 mL of blood sampled at 12, 20, and 30 weeks gestation (ultrasound confirmed gestation ±10 days) and in the 24 hours before delivery.

Statistical analysis was performed with non-parametric tests. In the first study, the Mann-Whitney U test was used to compare pre- and post-delivery values in the PE and NT groups. In the longitudinal study, the area under the curve (AUC) was calculated for each woman at 12, 20, and 30 weeks and pre-delivery. The AUCs were then compared between the PE and NT groups at each of the time points and analyzed using the Mann-Whitney U test. Demographic patient data were compared with the unpaired Student’s t test for normally distributed data. The Spearman rank test was used to assess correlation with other clinical variables. P < 0.05 was considered statistically significant.

**Results**

The characteristics of the women in the pre- and post-delivery and longitudinal VEGF studies are outlined in Tables 1 and 2. All subjects were white except for 1 Asian woman with PE in the pre- and post-delivery study. It was possible to select controls for the pre- and post-delivery study of similar age, gestation, and mode of delivery due to the nature of the cross-sectional study. This matching was not possible in the longitudinal study where the mean gestation of delivery in the PE group was 34.7 weeks, significantly lower than in the GH and NT women (P < 0.0001).

The individual serum VEGF levels pre- and post-delivery for the PE (n = 20) and NT (n = 25) women are shown in the scatter plot (Figure 1). The pre-delivery median serum VEGF level in the PE group was 51.7 ng/mL, ~4 times the median level in the control group of 13.9 ng/mL (P < 0.0001). Serum VEGF levels fell rapidly within 24 hours of delivery in both the PE and NT groups, with median values of 3.8 ng/mL and 3.2 ng/mL respectively (P < 0.3). The women with the 2 highest VEGF levels in the predelivery PE group had thrombocytopenia, with platelet levels of <100 000/mL, although liver function tests were within normal limits. No other PE subject had thrombocytopenia or abnormal liver function tests.

Of the 400 women who consented to have blood samples taken at 12 weeks gestation, 23 (5.7%) developed PE, and 29 (7.2%) developed GH. However, only 10 PE, 10 GH, and 28 NT women had their blood samples taken at all the relevant gestations (ultrasound confirmed gestation ±10 days) and in the 24 hours before delivery. Unfortunately, many controls in the longitudinal study failed to have a pre-delivery blood sample taken and were therefore excluded from the final data analysis. A further 31 women were excluded from the study because of complications, including miscarriage, ante-partum hemorrhage, intrauterine growth retardation in the absence of PE, chorioamnionitis, and pre-term labor and delivery.

The median serum VEGF levels at 12, 20, and 30 weeks gestation and pre-delivery for the PE (n = 10), GH (n = 10), and NT (n = 28) groups are shown in Figure 2. The individual serum VEGF levels for each woman in the 3 groups at each time interval are shown in the scatter plots in Figure 3. At 12 weeks, there was no significant difference in the serum VEGF concentrations of the 3 groups (P > 0.3). At 20 weeks, there was a noticeable increase in the concentrations of VEGF in the PE group; it almost reached significance in comparison with the other groups (P < 0.052). At 30 weeks, however, the serum VEGF levels in the eventual PE group were significantly different from those of the GH and NT groups (P < 0.001). Immediately before delivery, serum VEGF con-
centrations were significantly elevated in the PE group versus concentrations in the GH and control groups ($P<0.0001$). The values were similar to those found in the pre-delivery group in the first study. None of the PE group in the longitudinal study showed any signs or symptoms of their condition at their 30-week antenatal visit. Although 1 subject was delivered at 31 $\pm$ 5 weeks because of PE, the remaining subjects were delivered between 32 $\pm$ 3 and 38 weeks gestation. This subject’s VEGF concentration at 30 weeks was 32.5 ng/mL, whereas the range for the PE group was 16.8 to 40.4 ng/mL. The highest VEGF concentration at 30 weeks gestation (40.4 ng/mL) was in a subject who developed severe PE at 32 $\pm$ 3 weeks gestation, with admission BP of 180/120 mm Hg and 4+ proteinuria but no thrombocytopenia. The VEGF concentrations at 30 weeks gestation in those developing PE between 35 and 38 weeks gestation ranged from 16.8 to 31.3 ng/mL. At 20 weeks, the highest levels of VEGF were from the subjects who developed PE at 31 $\pm$ 5 and 32 $\pm$ 3 weeks gestation. The GH and NT subjects were delivered from 36-41 $\pm$ 4 weeks gestation.

**Discussion**

In the studies reported in this paper, we have confirmed that serum VEGF concentrations are significantly elevated in

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PE (n=10)</th>
<th>GH (n=10)</th>
<th>NT (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>22.3 (4.9)</td>
<td>22.8 (4.5)</td>
<td>23.1 (3.8)</td>
</tr>
<tr>
<td>Weight at admission, kg</td>
<td>61.1 (4.1)</td>
<td>61.7 (5.2)</td>
<td>63 (6.7)</td>
</tr>
<tr>
<td>Gestation at delivery, weeks + days</td>
<td>34 $\pm$ 6(2 $\pm$ 2)*</td>
<td>39 $\pm$ 3 (1 $\pm$ 3)</td>
<td>39 $\pm$ 6 (1 $\pm$ 1)</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>2.6 (0.4)*</td>
<td>3.6 (0.4)</td>
<td>3.6 (0.3)</td>
</tr>
<tr>
<td>Number &lt;10th centile</td>
<td>2 (20%)*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td>159/103 (11.6/8.8)†</td>
<td>153/93 (11.8/5.2)</td>
<td>122/69 (11.4/7.9)</td>
</tr>
<tr>
<td>Plasma urate, mg/dL</td>
<td>6.1 (0.9)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>6 (60)</td>
<td>5 (50)</td>
<td>12 (42)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>4 (40)</td>
<td>5 (50)</td>
<td>16 (58)</td>
</tr>
<tr>
<td>Induction of labor (or elective c/section), n (%)</td>
<td>10 (100)</td>
<td>7 (70)</td>
<td>15 (53)</td>
</tr>
<tr>
<td>Normal vaginal delivery, n (%)</td>
<td>5 (50)</td>
<td>6 (60)</td>
<td>17 (60)</td>
</tr>
<tr>
<td>Caesarean section, n (%)</td>
<td>4 (40)</td>
<td>1 (10)</td>
<td>3 (11)</td>
</tr>
</tbody>
</table>

Values are mean (SD) unless otherwise indicated.

N/A = not applicable.

* $P<0.001$ vs GH and NT groups.

† $P<0.001$ vs NT group.

Figure 1. Scatter plot of individual antenatal and postnatal VEGF concentrations in PE and NT women at term. Bar indicates median. ***$P<0.0001$.

Figure 2. Median serum VEGF levels at 12, 20, and 30 weeks gestation and 24 hours predelivery in PE (n=10), GH (n=10) and NT (n=28) women. **$P<0.001$; ***$P<0.0001$. 
clinical PE. However, we have also shown that these elevated concentrations fall to levels similar to those of non-PE patients within 24 hours of delivery. This suggests that the main source of VEGF production lies with the fetus and placenta. Additionally, we have also demonstrated that concentrations of VEGF are significantly elevated in PE subjects for several weeks before the clinical manifestation of the condition. The concentrations of VEGF that occur before the clinical disease manifests may also be indicative of the timing of the onset with the highest levels at 20 and 30 weeks occurring in those who developed PE earliest.

In the last few years, attempts have been made to measure VEGF concentrations in PE, with conflicting results. Baker et al., using an immunofluorescent ELISA assay, demonstrated an elevation in some women with PE whereas in both NT pregnant and non-pregnant controls the levels were undetectable. Sharkey et al. and Kupferminc et al. also showed elevated levels in preeclamptic women with the use of a VEGF competitive enzyme immunoassay. However, Lyall et al. using a commercial ELISA assay, found that serum VEGF levels were decreased in both PE and pregnancy in general versus non-pregnant controls. Their finding that VEGF was not elevated in pregnancy is surprising because it is involved in both embryogenesis and placental formation. Protein binding of VEGF may cause problems with all VEGF assays, including the one used in this study. However, in response to the results of earlier reports, Anthony et al. developed a polyclonal antibody radioimmunoassay to VEGF, which addressed many of the potential problems of the ELISA in the detection of bound VEGF in pregnancy. Evans et al., using this assay, subsequently demonstrated that VEGF was detectable in increasing levels from early pregnancy.

Recently, it has been shown that the VEGF receptor flt-1 is found in the blood of pregnant women but not in non-pregnant women or men: in the serum it is known as soluble flt-1 (sflt-1). Flt-1 is normally located across the cell membrane on endothelial cells and extravillous trophoblast. It binds VEGF with high affinity and prevents VEGF action on vascular endothelial cells. Histological studies have demonstrated sflt-1 production from both extravillous trophoblast and endothelial cells. The presence of sflt-1 in the blood may affect VEGF binding and, hence, VEGF measurement by assays. The development of assays to measure serum sflt-1 concentration and their use in conjunction with those for VEGF may help clarify the differences seen in VEGF concentrations in different assay systems. It is unknown why sflt-1 is present in the maternal circulation in pregnancy. Perhaps it is a protective response by the placenta to mop up the excessive serum VEGF levels circulating in maternal blood. If so, an imbalance of VEGF/sflt-1 may be important in the pathophysiology of PE. Interestingly, heparin greatly increases the binding of VEGF to flt-1 and has been advocated as a treatment of PE.

The results of our pre- and post-delivery study show that serum VEGF concentrations fall rapidly after delivery. This
may suggest that the fetus and the placenta are the main sources of VEGF production in PE. In theory, preeclamptic placentae are under-perfused and hypoxic. This is likely to be due to deficient spiral artery invasion by trophoblast in PE. Thus, the vessels fail to convert from high-pressure low-capacitance to low-pressure high-capacitance vessels. As a result, they are prone to acute atherosis, necrosis, and infarction.20 As the pregnancy progresses, it is thought that the chronic underperfusion of the placenta results in hypoxia-driven production of VEGF by the placental trophoblasts. However, Cooper et al21 showed VEGF production in the PE placenta was lower than in the NT placenta and suggested that VEGF may be released in PE from damaged endothelium.21 It is possible that VEGF is produced, at least in part, by damaged endothelium in PE.

Our study did not include any women who developed post-partum PE/eclampsia or who developed a worsening clinical outlook after delivery. A larger study including such patients might provide further elucidation of the site of VEGF production in PE. If serum VEGF were elevated post-delivery in such patients, this would suggest that VEGF is produced by the endothelium, because it could no longer be produced by the placenta. It may be possible in the future to produce poor spiral artery invasion in the placenta of a pregnant animal model. If so, the subsequent placental VEGF and serum concentrations would be of great interest in answering the question regarding the site of VEGF production.

Elevation of serum VEGF may allow, either alone or in combination with other biochemical markers (such as inhibin A or fibronectin), early identification of those pregnancies likely to result in PE.22,23 Such a test would enable identification of a high-risk group that may benefit from close monitoring, steroid administration, and anti-hypertensive therapy. VEGF antagonists such as sflt-1 may counteract the endothelial effects of VEGF and thus prevent or ameliorate the disease and allow progression of the pregnancy. Further work including larger clinical studies to enable determination of the positive and negative predictive values of the serum concentrations and the role of sflt-1 are indicated. In vivo studies may assist in determining the source of VEGF production and its effects when elevated in the blood of pregnant animals.

In summary, serum levels of VEGF are elevated both during and before the manifestation of clinical PE. However, larger studies of VEGF and sflt-1 levels in pregnancy are indicated. In vivo monitoring, steroid administration, and anti-hypertensive therapy. VEGF antagonists such as sflt-1 may counteract the endothelial effects of VEGF and thus prevent or ameliorate the disease and allow progression of the pregnancy. Further work including larger clinical studies to enable determination of the positive and negative predictive values of the serum concentrations and the role of sflt-1 are indicated. In vivo studies may assist in determining the source of VEGF production and its effects when elevated in the blood of pregnant animals.

In summary, serum levels of VEGF are elevated both during and before the manifestation of clinical PE. However, larger studies of VEGF and sflt-1 levels in pregnancy are necessary to confirm the potential role of these substances in the prediction of PE.

Acknowledgments
We are grateful to the Northern Ireland Mother and Baby Appeal (NIMBA) and the Royal Maternity Hospital for a Research Fellowship.

References
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Hypertension, 2000;36:965-969
doi: 10.1161/01.HYP.36.6.965

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