Relationship between maternal serum vascular endothelial growth factor concentration in early pregnancy and fetal and placental growth

T.Wheeler1,3, P.W.Evans1, F.W.Anthony1, K.M.Godfrey2, D.T.Howe1 and C.Osmond2

1Obstetrics and Gynaecology and 2Medical Research Council Environmental Epidemiology Unit, University of Southampton, Southampton SO16 5YA, UK
3To whom correspondence should be addressed at: Obstetrics and Gynaecology (815), University of Southampton, Princess Anne Hospital, Coford Road, Southampton SO16 5YA, UK

Vascular endothelial growth factor (VEGF) has important effects on endothelial cells increasing cell proliferation, permeability and nitric oxide production; concentrations of VEGF in the maternal serum increase during the first 10 weeks of pregnancy. In this study, the relationship of maternal serum VEGF with maternal health during pregnancy and with fetal and placental size at mid-pregnancy and at term was investigated. Serum was obtained from 539 Caucasian women with singleton pregnancies between 8 and 20 weeks of pregnancy (mean 14 weeks). Total serum VEGF concentrations were measured by direct competitive radioimmunoassay. Fetal size and placental volume were measured by ultrasound between 16 and 20 weeks gestation. Birthweight, placental weight and anthropometric measurements of the baby were obtained after delivery. Serum VEGF concentrations were found to be higher in women with a lower weight before pregnancy ($P = 0.01$) and in those carrying a female fetus ($P = 0.002$). VEGF concentrations were positively correlated with placental volume ($r = 0.17$, $P = 0.0001$) but not with fetal size between 16 and 20 weeks gestation. Serum VEGF concentrations were positively correlated with both birthweight ($r = 0.10$, $P = 0.02$) and placental weight at delivery ($r = 0.13$, $P = 0.003$). The data presented support the view that VEGF may be one of the factors involved in mediating the maternal cardiovascular adaptation to pregnancy.

Key words: birthweight/placental volume/VEGF

Introduction

Vascular endothelial growth factor (VEGF) is distributed widely throughout the tissues of the adult and fetus (Shifren et al., 1994). Although only one of a large number of factors which can induce angiogenesis, VEGF has a key role in fetal development. Gene deletion studies have shown that embryonic mice unable to produce VEGF or deficient in VEGF receptors fail to develop a normal vasculature and abort (Shalaby et al., 1995; Carmeliet et al., 1996). Angiogenesis is also important in specific maternal tissues such as the endometrium. The importance of vascularization at this site is shown by experiments in which pregnant mice given an angiogenesis inhibitor around the time of implantation have impaired endometrial vascularization, leading to defective placentation and ultimately to resorption of the embryos (Klauber et al., 1997).

We have developed a radioimmunoassay which measures both the bound and free forms of VEGF in human serum (Anthony et al., 1997), and have found that the concentration of VEGF in maternal serum increases early in the first trimester (Evans et al., 1997). The source of this increase is unknown, but possible sites include the corpus luteum, the uterine endometrium and the placenta because of their increased angiogenic activity during pregnancy; expression of VEGF mRNA has been demonstrated in these tissues (Charnock-Jones et al., 1993; Anthony et al., 1994; Kamat et al., 1995). In addition to its role in stimulating angiogenesis, VEGF has been shown to up-regulate the production of nitric oxide (NO) by endothelial cells (Van der Zee et al., 1997). NO is a potent vasodilator, whose production is increased from early in pregnancy (Williams et al., 1997). Vasodilatation is a critical event in early pregnancy, affecting not only the maternal vessels adjacent to the site of implantation (Nanaev et al., 1995) but also reducing peripheral resistance in the maternal systemic circulation as a whole. The latter change is believed to initiate the increases in cardiac output and plasma volume that characterize early pregnancy (Duvekot et al., 1993); these changes have important influences on fetal growth (Rosso et al., 1993). The production of VEGF by vascular smooth muscle cells is increased by oestrogen (Karas et al., 1996), thus VEGF could reach the maternal circulation through the systemic vasculature.

To investigate further the possible role of VEGF in early pregnancy, its concentration was measured in maternal serum around 14 weeks gestation and the serum concentrations were correlated with measures of fetal and placental size obtained at mid-pregnancy by ultrasound and following delivery by weighing and anthropometry.

Materials and methods

The study cohort has been described previously (Godfrey et al., 1996a). In summary, over a 1-year period (1992/93) all 655 Caucasian women aged 16 years or more who registered with two obstetricians at the Princess Anne Hospital, Southampton were approached. Women with multiple pregnancies, diabetes and those who had undergone fertility treatment were excluded. Twelve miscarried or had a termination of pregnancy; seven delivered outside the hospital. 596 (94%) of the remaining 636 gave informed consent to participate in the study, which was approved by the local ethics committee. The mothers
The average serum VEGF concentration was 3.20 µg/l; the concentrations were positively skewed and were not correlated with gestation. Further details are given in Table I. Before comparison with other variables, the VEGF concentrations were normalized by taking square roots.

Details of the ultrasound measurements of fetal size and placental volume made between 16 and 20 weeks of pregnancy are given in Table I. All the ultrasound measurements were positively correlated with gestational age and adjusted for this effect by linear regression before comparison with other variables. Birthweight, placental weight and the anthropometric measurements made following delivery are shown in the Table I for the 504 babies born at greater than 37 weeks gestation.

Before correlation with serum VEGF concentrations, the variables were normalized by taking square roots.

Serum VEGF was measured by a direct competitive radioimmunoassay (Anthony et al., 1997). Briefly, recombinant human VEGF165 was labelled with 125I to act as tracer, and known quantities of unlabelled VEGF were used to construct a standard curve. Duplicate standards, controls and unknown serum samples were incubated overnight with tracer and rabbit polyclonal antiserum. Following a 30-min incubation with donkey anti-rabbit coated cellulose suspension, the mixture was suspended in 1 ml of distilled water, and centrifuged. After decanting, radioactivity of the pellets was measured by gamma counter and analysed by computer by the RIA-CALC, LKB-Wallac package. The inter-assay coefficient of variation for the VEGF assay was 12.9% for pregnancy serum samples of mean concentration 5.90 µg/l. The sensitivity of the assay was 0.1 µg/l.

were visited at home shortly after recruitment and details obtained about their pre-pregnancy weight and smoking habit. Ninety percent of the women were able to ascertain their own birthweight by contacting members of their family.

Maternal serum was collected at the first antenatal visit between 8 and 20 weeks of pregnancy (average 14 weeks) and stored at −40°C. An aliquot was available for the measurement of VEGF concentration in 539 women (90% of those who agreed to take part). There were no significant differences between the pre-pregnant maternal weights or between the birthweights of the offspring of the subjects who were or were not included in the study. 54% of the women were nulliparous or between the birthweights of the offspring of the subjects who were

Table I. Maternal, fetal and neonatal data. Correlation with maternal serum vascular endothelial growth factor (VEGF)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum VEGF (µg/l)</td>
<td>539</td>
<td>3.20</td>
<td>1.18</td>
<td>0.80</td>
<td>9.53</td>
<td>–0.07</td>
<td>0.10</td>
</tr>
<tr>
<td>Maternal characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>539</td>
<td>26</td>
<td>4.9</td>
<td>16</td>
<td>43</td>
<td>–0.07</td>
<td>0.10</td>
</tr>
<tr>
<td>Weight before pregnancy (kg)</td>
<td>530</td>
<td>61.3</td>
<td>12.0</td>
<td>38</td>
<td>122</td>
<td>–0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>535</td>
<td>163</td>
<td>6.1</td>
<td>137</td>
<td>180</td>
<td>–0.04</td>
<td>0.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>526</td>
<td>23</td>
<td>4.3</td>
<td>14.0</td>
<td>44.8</td>
<td>–0.11</td>
<td>0.02</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>485</td>
<td>3291</td>
<td>58.5</td>
<td>109</td>
<td>454</td>
<td>0.17</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ultrasound measurements at 16–20 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental volume (ml)</td>
<td>503</td>
<td>245</td>
<td>58.5</td>
<td>109</td>
<td>454</td>
<td>0.17</td>
<td>0.0001</td>
</tr>
<tr>
<td>Femur length (mm)</td>
<td>536</td>
<td>28.1</td>
<td>3.1</td>
<td>18</td>
<td>40</td>
<td>0.01</td>
<td>0.9</td>
</tr>
<tr>
<td>Head circumference (mm)</td>
<td>513</td>
<td>152</td>
<td>12.2</td>
<td>121</td>
<td>208</td>
<td>0.03</td>
<td>0.5</td>
</tr>
<tr>
<td>Abdominal circumference (mm)</td>
<td>505</td>
<td>136</td>
<td>13.1</td>
<td>99</td>
<td>197</td>
<td>0.04</td>
<td>0.3</td>
</tr>
<tr>
<td>Biparietal diameter (mm)</td>
<td>536</td>
<td>43</td>
<td>3.6</td>
<td>31</td>
<td>58</td>
<td>–0.01</td>
<td>0.7</td>
</tr>
<tr>
<td>Neonatal measurements (&gt;37 weeks gestation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestation at delivery (days)</td>
<td>504</td>
<td>281</td>
<td>9.3</td>
<td>259</td>
<td>304</td>
<td>0.06</td>
<td>0.2</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>504</td>
<td>3435</td>
<td>478</td>
<td>2050</td>
<td>5020</td>
<td>0.10</td>
<td>0.02</td>
</tr>
<tr>
<td>Placental weight (g)</td>
<td>489</td>
<td>532</td>
<td>119</td>
<td>240</td>
<td>1048</td>
<td>0.13</td>
<td>0.003</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>494</td>
<td>35.1</td>
<td>1.3</td>
<td>31.2</td>
<td>39.4</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>494</td>
<td>33.7</td>
<td>1.8</td>
<td>29.1</td>
<td>39.5</td>
<td>0.07</td>
<td>0.11</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>488</td>
<td>50.2</td>
<td>2.0</td>
<td>43.4</td>
<td>55.8</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>HC/AC ratio (%)</td>
<td>494</td>
<td>104.3</td>
<td>4.2</td>
<td>93.1</td>
<td>117.8</td>
<td>–0.03</td>
<td>0.5</td>
</tr>
<tr>
<td>Ponderal index (kg/m³)</td>
<td>488</td>
<td>27.1</td>
<td>2.1</td>
<td>21.0</td>
<td>33.7</td>
<td>0.03</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Correlation (r) and significance (P) with serum VEGF.

*(Head circumference/abdominal circumference)×100.
The correlations between maternal VEGF concentrations and the maternal, fetal and neonatal characteristics are given in Table I. The VEGF measurements were inversely correlated with maternal weight and body mass index (BMI) before pregnancy but not to maternal height or maternal birthweight. Serum VEGF was not correlated with maternal parity or with maternal smoking. There was no significant difference in the mean serum VEGF concentration in the 16 women who developed pre-eclampsia compared to those who were normotensive (2.81 versus 3.11 µg/l, P = 0.3).

Serum VEGF was positively correlated (r = 0.17, P = 0.0001) with the ultrasound estimations of placental volume between 16 and 20 weeks gestation (Figure 1). Though placental volume was strongly correlated with all the fetal dimensions (P < 0.0001), none of the measures of fetal size was associated with VEGF concentration (Table I). VEGF concentrations were positively correlated with placental weight at delivery (r = 0.13, P = 0.003; Figure 2) and birthweight (r = 0.10, P = 0.02; Figure 3) and with the anthropometric measurements of the baby (Table I). Mean serum VEGF concentration in women carrying female fetuses was significantly higher than in women carrying male fetuses (3.26 versus 2.96 µg/l, P = 0.002). Multiple regression allowing for fetal sex and maternal weight did not significantly change the relationships between serum VEGF and placental volume (at 16–20 weeks), birthweight or placental weight at delivery.

Discussion
It has previously been shown that maternal serum VEGF concentrations increase during the first trimester (Evans et al., 1997, 1998). The current study shows that serum VEGF concentrations remain elevated up to 20 weeks of pregnancy and that concentrations are positively correlated with placental volume at mid-pregnancy and with placental and fetal weight at delivery. The inverse relationship between maternal pre-pregnancy weight and serum VEGF concentration parallels the associations between maternal weight and serum levels of human chorionic gonadotrophin (HCG) and progesterone (Evans et al., 1997) and may reflect haemodilution, heavier women having a larger plasma volume; allowance for maternal weight did not alter the correlations demonstrated in this study. Since VEGF is not believed to have an endocrine role, its presence in the circulation is likely to represent an increase in local production elsewhere in the body, which spills into the circulation. It has previously been shown that the contribution of the corpus luteum to circulating levels of VEGF is low in comparison to other potential sources (Evans et al., 1998). These other sources include the uterus and placenta, sites where VEGF transcription has been shown to increase throughout pregnancy (Ni et al., 1997). Another study has also found an
excess of VEGF binding protein to be present in the serum during pregnancy (Anthony et al., 1997); it is possible that this protein is the truncated soluble form of the VEGF fli-t receptor, which has recently been shown to be produced by the placenta (Clark et al., 1997). This receptor would be likely to inactivate the circulating VEGF (Kendall et al., 1996).

The increase in maternal serum VEGF may result from VEGF production by vascular smooth muscle cells in the maternal systemic circulation following oestrogen stimulation (Karass et al., 1996). VEGF increases the production of NO by endothelial cells (Van der Zee et al., 1997) and may be part of the cellular mechanism responsible for the peripheral vasodilatation, which characterizes early pregnancy. The plateau of VEGF concentrations around 10 weeks of pregnancy coincides with the decrease in maternal vascular resistance which reaches its nadir at the same time (Robson et al., 1989). The increases in maternal cardiac output and plasma volume, both initiated by the decrease in peripheral resistance in early pregnancy (Duvekot et al., 1993), are positively linked with fetal growth (Rosso et al., 1993) and may account for the correlation of VEGF with birthweight. In discussing the possible roles for VEGF during pregnancy it is important to note that the associations found in this study have low correlation coefficients and causality is not necessarily implied.

A number of studies show that VEGF may have an important role in the development of the uterine circulation during pregnancy. NO synthase is present in syncytiotrophoblast cells (Myatt et al., 1993) and VEGF has been shown to up-regulate NO production by these cells (Ahmed et al., 1997); it has been suggested that local NO production may improve blood flow within the intervillous space by reducing platelet and leukocyte adhesion to trophoblast cells (Myatt et al., 1993). Nitric oxide has also been shown to promote vasodilatation of the uterine arcuate artery in rats, an effect initiated in part through VEGF (Ni et al., 1997). Thus perfusion of the intervillous space may be influenced by a number of local mechanisms involving VEGF. This proposal is supported by the findings of this study, which show a stepwise increase in placental volume (a measurement which includes the maternal blood within the intervillous space) as maternal serum VEGF increases (Figure 1).

In late pregnancy, maternal serum VEGF concentrations are increased in women who have established pre-eclampsia (Sharkey et al., 1996; Kupferminc et al., 1997; Brockelsby et al., 1998); however, in early pregnancy, no difference was found in VEGF levels in the 16 women who subsequently became pre-eclamptic. The vascular changes which result in an increase in serum VEGF in pre-eclampsia may be different to those which influence VEGF production in early pregnancy; for example endothelial function is altered in pre-eclampsia (McCarthy et al., 1993) and experimental damage to the endothelium has been shown to increase the production of VEGF by vascular smooth muscle cells (Tsurumi et al., 1997). It was also found that maternal serum VEGF concentrations were significantly higher (P < 0.002) in women who were carrying a female fetus. A previous study has shown that urinary nitrates and nitrites are linked in a similar way to fetal sex (Garmendia et al., 1997); these findings support the links proposed here between VEGF and nitric oxide production. Other studies (Obiekwe and Chard, 1982; Steier et al., 1989) have shown that levels of HCG are increased in women who are carrying a female fetus and significant correlations have previously been noted between the levels of HCG and VEGF in the maternal serum in early pregnancy (Evans et al., 1997, 1998).

In summary, it was found that maternal serum VEGF concentrations, measured in the first half of pregnancy are correlated with the growth of the fetus and placenta. It can be postulated that these findings may be partly explained by the influence of VEGF on vascular nitric oxide production. Myographic studies examining the influence of VEGF on isolated blood vessels obtained from pregnant and non-pregnant subjects may clarify the role of VEGF in early pregnancy.

Acknowledgements

This research was supported by Southampton University Medical School, the Solent Subfertility Trust, the Board of Health of the States of Guernsey (through the Wessex Medical Trust) and the Medical Research Council. We are grateful to Genentech for the supply of VEGF reagents.

References


Received on October 1, 1998; accepted on February 15, 1999