Key words: Angiogenic markers, pre-eclampsia.

Introduction

Every year, 536,000 women die from pregnancy-related causes. More than 80 percent of maternal deaths worldwide are due to five direct causes: hemorrhage, sepsis, unsafe abortion, obstructed labor and hypertensive disease of pregnancy. The latest report from the Confidential Enquiries into Maternal Deaths (UK) lists eclampsia and pre-eclampsia as the second most common cause and showed an increase from the previous report. The latest statistics from the Philippine Obstetrical and Gynecological Society (2006) listed hypertension as causing 143/545 (26.24%) maternal deaths. Further broken down, hypertension deaths were preeclampsia (50), eclampsia (66), pre-existing hypertension (8), chronic hypertension with pre-eclampsia (8) and HELLP syndrome (11). Clearly, therefore, any attempt to curb maternal and perinatal mortality and morbidity due to hypertensive states will result not only in less loss of lives but also in less deprivation of financial and material resources. This paper presents an update on a new pseudo-vasculogenesis theory and its possible application as diagnostic markers in the prediction and prevention of pre-eclampsia.

Pathophysiology of pre-eclampsia

Numerous theories have been advanced regarding the cause(s) of PIH and the reader is referred to the book of Chesley for a historical review of these.

"...Everyone from allergists to zoologists has proposed hypotheses and suggested radical therapies based upon them such as mastectomy, oophorectomy, renal decapsulation, trephination, alignment of the patient with the earth's magnetic field with her head pointing toward the North Pole and all sorts of medical regimens."

(Leon Chesley, 1971)

Current data indicate that placental ischemia and trophoblastic hypoperfusion with endothelial dysfunction is the most consistent change in preeclampsia and is believed to be the pivotal insult in this disease.

Pseudo-vasculogenesis Theory

A pathophysiological study has examined the hypothesis that placental ischemia occurs at an early stage and upregulates placental production of a soluble anti-angiogenesis protein called soluble fms-like tyrosine kinase 1 (sFlt1) that leads to maternal endothelial dysfunction and its clinical sequelae of hypertension, proteinuria, and edema. sFlt1 acts as a potent antagonist to vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) which help to reduce vascular tone and blood pressure (pro-angiogenesis).

The study further showed the following findings:

1. total serum sFlt1 was nearly 5x higher in those with severe preeclampsia than in normotensive women, and this difference was not explained by earlier gestational age.
2. serum ELISA levels of free VEGF and PIGF were significantly reduced in the presence of recombinant sFlt1.
3. Angiogenesis, as reflected by endothelial tube formation, was inhibited by serum from women with preeclampsia, and an analogous effect was noted in vitro when sFlt1 to was added serum from normotensive women. Adding VEGF and PIGF to preeclamptic serum restored endothelial tube formation. An in vitro assay for microvascular reactivity showed that sFlt1 blocked the dose-dependent increase in vasodilation produced by VEGF or PIGF.
4. Most importantly, recombinant adenovirus encoding the murine sFlt1 gene product produced significant hypertension and heavy albuminuria when injected into pregnant rats. Histopath examination in the pregnant rats treated with sFlt1 showed renal glomeruli were enlarged and capillary loops were occluded by swollen, hypertrophied endocapillary cells. These findings suggest a cause and effect relationship that excessive placental production of sFlt1 contributes...
Angiogenic markers in pre-eclampsia

Vol. 31, No. 1, 2009

Another protein factor in pseudo-vasculogenesis is soluble endoglin. It is upregulated in syncytiotrophoblasts and its levels are exaggerated during and, significantly, prior to the onset of preeclamptic symptoms. Levine et al have recently demonstrated a 2-3x endoglin increase in preterm and term pregnancy, respectively, compared to non-pregnant states. Significantly, mild preeclampsia, severe preeclampsia, and HELLP syndrome are accompanied by a further three-, five-, and ten-fold increase in circulating endoglin, respectively. These elevated endoglin levels closely parallel increases in sFlt-16.

What causes the upregulation of sFlt1 and other anti-angiogenesis proteins? Fisher, et al 7 offers an explanation that placental hypoxia (decreased oxygen) serves as the driving force for the phenomenon of “pseudovasculogenesis” (Figure 1). This process can be described as follows:

1. The early stages of placental development take place in a relatively hypoxic environment that favors cytotrophoblast proliferation rather than differentiation along the invasive pathway. Accordingly, this cell population (green cells) rapidly increases in number as compared with the embryonic lineages.

2. As development continues, cytotrophoblasts (dark green cells) invade the uterine wall and plug the maternal vessels, a process that helps maintain a state of physiological hypoxia. As indicated by the blunt arrows, cytotrophoblasts migrate farther up arteries than veins.

3. By 10 to 12 weeks of human pregnancy, blood flow to the intervillous space begins. As the endovascular component of cytotrophoblast invasion progresses, the cells migrate along the lumina of spiral arterioles, replacing the maternal endothelial lining. Cytotrophoblasts are also found in the smooth muscle walls of these vessels. In normal pregnancy the process whereby placental cells remodel uterine arterioles involves the decidual and inner third of the myometrial portions of these vessels. As a result, the diameter of the arterioles expands to accommodate the dramatic increase in blood flow that is needed to support rapid fetal growth later in pregnancy. It is likely that failed endovascular invasion leads, in some cases, to abortion, whereas an inability to invade to the appropriate depth is associated with preeclampsia and a subset of pregnancies in which the growth of the fetus is restricted.

Figure 1. Pseudovasculogenesis and placental hypoxia (Fisher et al)
Clinical studies on angiogenic markers

sFlt-1. To evaluate gestational patterns, Levine et al performed a cross-sectional analysis of serum obtained within gestational-age intervals of four to five weeks (Figure 3A). The sFlt-1 concentrations in controls remained constant until 33 to 36 weeks of gestation, when they increased by approximately 145 pg per milliliter per week until labor and delivery. In the women in whom preeclampsia later developed, but before the onset of preeclampsia, the concentrations began to increase at 21 to 24 weeks of gestation, with a steeper increase at 29 to 32 weeks. When they compared the sFlt-1 concentrations at different stages of pregnancy in specimens from women in whom preeclampsia developed, they found that among women with specimens drawn when their fetuses were in the same gestational-age interval, those who already had clinical preeclampsia had significantly higher concentrations than those who did not (Figure 3A).

The concentrations of sFlt-1 were increased in women who later had preeclampsia beginning 11 to 9 weeks before the onset of preeclampsia. Five weeks before the onset of preeclampsia, the average concentration in women in whom preeclampsia developed was similar to the concentrations in controls at term, but after this point the sFlt-1 concentration in the women with preeclampsia was higher (Figure 3B).

Placental growth factor

The PlGF concentrations in the controls increased during the first two trimesters, peaked at 29 to 32 weeks, and decreased thereafter. The PlGF concentrations in the women with preeclampsia followed a similar pattern but were significantly lower than those in the controls from 13 to 16 weeks onward (Figure 4A).

Similar to s-Flt-1, the PlGF concentrations began to decrease 11 to 9 weeks before the onset of preeclampsia, with substantial reductions during the 5 weeks before the onset of hypertension or proteinuria (Figure 4B).

The key points of these landmark articles are:

- The pro-angiogenic markers, PlGF and VEGF, both decrease with pre-eclampsia compared to normotensive women.

A summary diagram shows the current thinking that pre-eclampsia may be considered a 2-stage disease (Figure 2).

![Figure 2. Pre-eclampsia a 2-stage disease](image-url)
Figure 3A. Mean sFlt-1 concentration and gestational age

Figure 4A. Mean PIGF concentration and gestational age
The anti-angiogenetic maker, sFlt-1, increases with preeclampsia compared to normotensive controls.

The decrease in PIGF and the increase in s-Flt-1 are evident 5 weeks before the onset of clinical pre-eclampsia.

There have been numerous articles which have validated these initial findings on angiogenetic markers and will not be mentioned here.

Future directions

Some articles have also done studies on other markers (soluble endoglin, PAPP-A, PP-13 or placental protein-13, podocyturia) and are worth remembering.

The use of ratios between the pro- and the anti-angiogenetic markers have also been undertaken.

Studies using the combination of angiogenetic marker testing in the 1st trimester combined with uterine artery Doppler investigation (presence of a diastolic notch and pulsatility indices) are certainly worth looking into.

References