Preeclampsia

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Dedication

To the Department of Nephrology at NYU
Stop worrying, you will not develop preeclampsia
Definitions

Categories

Pathophysiology:
  • Placental abnormalities, secretion of angiogenic factors, systemic endothelial response

Diagnosis

Treatment

Prognosis
Definitions

- **Preeclampsia:**
  - Proteinuria and HTN after 20 weeks of gestation.

- **Chronic HTN:**
  - HTN before pregnancy, before 12 weeks of pregnancy, or persisting longer than 12 weeks postpartum

- **Gestational HTN:**
  - HTN without proteinuria developing in the latter part of pregnancy and resolving within 12 weeks postpartum

- **Chronic HTN Masked During Pregnancy:**
  - HTN without proteinuria developing in the latter part of pregnancy and not resolving 12 weeks postpartum

- **Eclampsia:**
  - Grand mal seizures in a woman with gestational HTN or preeclampsia
Categories

- **Timing:**
  - Early: <34 weeks
  - Late: ≥ 34 weeks

- **Severity:**
  - Mild: Does not meet the criteria for severe
  - Severe
Severity Criteria

- New onset Proteinuria and HTN and at least one of the following:
  - Symptoms of CNS dysfunction
  - Symptoms of liver capsule distension
  - Hepatocellular Injury
  - Severe Blood Pressure Elevation:
    - Systolic > 160 and diastolic > 110 on at least 2 occasions at least 6 hours apart
  - Thrombocytopenia:
    - < 100,000
  - Proteinuria:
    - > 5 grams
  - Severe fetal growth restriction
  - Pulmonary edema or cyanosis
  - CVA
Abnormalities in Placental Vasculature

- Antiangiogenic factors are secreted into the maternal circulation

- Systemic Maternal Endothelial Dysfunction
Abnormalities in Placental Vasculature
Normal Placental Development

- Extravillous cytotrophoblasts enter the lining of the uterus between 6 and 18 weeks
- The cytotrophoblasts meet the spiral arteries, which enlarge to form high capacitance blood vessels
- These blood vessels help supply the placentas needs after 20 weeks
The floating villi (FV) are in the intervillous space in direct contact with the maternal blood. In normal pregnancy, invasive cytotrophoblasts (CTB) form cell columns (Zone II/III) and invade maternal decidua and vasculature (Zone IV). During the differentiation along the invasive path, the cytotrophoblasts dramatically alter their expression of various molecules, such as integrins. In preeclampsia, the invasive cytotrophoblasts fail to differentiate along the invasive pathway and do not gain access to spiral arteries.

Courtesy of Kee-Hak Lim, MD.
Extravillous trophoblast cells do not meet the spiral arteries

- Pathologic examination of placentas in preeclampsia demonstrate
  - placental infarcts
  - sclerotic narrowing of the arteries and the arterioles
  - Diminished endovascular invasion by cytotrophoblasts
  - Inadequate remodeling of the spiral arteries
Abnormalities in Placental Development

- **No Pseudovasculogenesis**
  - Cytotrophoblasts do not change adhesion molecules from epithelial cells (integrin alpha 6/beta 1, alphav/beta5, and E-cadherin) to endothelial cells (integrin alpha1/beta 1, alphav/beta3, and VE-cadherin)

- **Immune Abnormalities:**
  - EVT express HLA C, E, and G: HLA-C signals paternal alloantigens.
  - NK cells have KIR (Killer immunoglobulin receptors) that recognize EVT HLAs and are either inhibitory or stimulatory.
  - Preeclampsia is more common in women who are homozygous for the inhibitory haplotypes on the KIRs
  - The effect is strongest in fetuses homozygous for HLA-C2
Antiangiogenic factors are secreted into the maternal circulation

sFlt and soluble Endoglin
sFlt (soluble fms-like tyrosine kinase 1) is a splice variant of the VEGF receptor flt-1.

- Acts as an antagonist to VEGF and PlGF
Maynard: Determine if sFlt could be responsible for the clinical manifestations of preeclampsia

- **Experiment 1:**
  - Determine if expression of sFLT-1 mRNA is higher in preeclamptic vs. control women, determine if serum sFlt-1 levels is higher in preeclamptic vs. control women
  - 4 samples from the placentas of women with mild and severe preeclampsia
  - Northern blot done, showing higher sFlt mRNA in preeclamptic patients
  - Serum was collected from pregnant women at delivery and 48 hours after delivery
  - The level of sFlt-1 was higher in the preeclamptic women than the controls, and normalized 48 hours after delivery.
Figure 1
mRNA and protein expression of sFlt1 in preeclampsia. (a) mRNA expression of placental sFlt1 from three patients with preeclampsia (P1, P2, and P3) and three normotensive term pregnancies (N1, N2, and N3) were determined by Northern blot analysis. The higher band (7.5 kb) is the full-length Flt1 mRNA, and the lower, more abundant band (3.4 kb) is the alternatively spliced sFlt1 mRNA. GAPDH is included as a loading control, and the location of 28S is indicated by an arrowhead. Patients P1 and P2 had severe preeclampsia, whereas patient P3 had mild preeclampsia. (b) ELISA was performed for sFlt1 on serum from patients with mild preeclampsia (PE), severe preeclampsia and from normotensive pregnant women at term (normal) as described in Table 1. Patients with preterm deliveries were included as additional controls to rule out changes due to gestational age. The numbers of patients tested are shown in parentheses on the x-axis. Serum samples were collected before delivery (t = 0) and 48 hours after delivery (t = 48). *P < 0.05 and **P < 0.01 as compared with normotensive controls.
Experiment 2:

- Determine if free VEGF and PlGF levels are decreased in patients with preeclampsia.

- Free VEGF and PlGF levels were determined for the control and preeclamptic patients at delivery.

- Both the VEGF and PlGF levels of preeclamptic patients were reduced.
Experiment 3:

- Determine if sFlt-1 impairs angiogenesis.
- Human umbilical vein cells were treated with 5% patient serum, plated, and incubated to assess for tube formation.
- Serum from normal patients allowed endothelial tube formation, and serum from patients with preeclampsia inhibited endothelial tube formation.
- Serum of preeclamptic women 48 hours after delivery allowed endothelial tube formation.
- sFlt-1 was added to the serum of the control patients, and then endothelial tube formation didn’t occur.
- Exogenous VEGF and PlGF was added to the serum for the preeclamptic patients at delivery, and endothelial tube formation occurred.
Figure 3
Preeclampsia is an antiangiogenic state due to excess sFlt1. Endothelial tube assay was performed using serum from four normal pregnant controls and four patients with preeclampsia before and after delivery. A representative experiment from one normal control and one patient with preeclampsia is shown. (a) $t = 0$ (5% serum from a normal pregnant woman at term). (b) $t = 48$ (5% serum from a normal pregnant woman 48 hours after delivery). (c) $t = 0$ plus exogenous sFlt1 (10 ng/ml). (d) $t = 0$ (5% serum from a preeclamptic woman before delivery). (e) $t = 48$ (5% serum from a preeclamptic woman 48 hours after delivery). (f) $t = 0$ plus exogenous VEGF (10 ng/ml) and PI GF (10 ng/ml). The tube assay was quantified, and the mean tube length $\pm$ SEM in pixels is given at the bottom of each panel for all the patients analyzed. Recombinant human VEGF, human PI GF, and human sFlt1-Fc were used for the assays.
Experiment 4:

- Determine if sFlt-1 can cause HTN and proteinuria in vivo
- Recombinant adenovirus with sFlt-1 was injected into the tails of pregnant (have VEGF and PlGF) and non-pregnant rats (have VEGF only).
- Both groups of rats developed proteinuria and HTN.
- Adenovirus containing Flk1, which only antagonizes VEGF, was then injected into the tails of pregnant and nonpregnant rats.
- Pregnant rats did not develop HTN and proteinuria (because PlGF was not affected), whereas nonpregnant rats did (because they don’t have PlGF)
**Table 2**  
Blood pressure and proteinuria in rats

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<th>Mean arterial pressure (mmHg)</th>
<th>Urine albumin/creatinine ratio (µg/mg)</th>
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<tr>
<td>Fc (pregnant)</td>
<td>5</td>
<td>75 ± 11</td>
<td>62 ± 21</td>
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<td>sFlt1 (pregnant)</td>
<td>4</td>
<td>109 ± 19&lt;sup&gt;A&lt;/sup&gt;</td>
<td>6923 ± 658&lt;sup&gt;B&lt;/sup&gt;</td>
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<tr>
<td>sFlk1-Fc (pregnant)</td>
<td>4</td>
<td>73 ± 15</td>
<td>50 ± 32</td>
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<tr>
<td>Fc (nonpregnant)</td>
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<td>89 ± 6</td>
<td>138 ± 78</td>
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<tr>
<td>sFlt1 (nonpregnant)</td>
<td>6</td>
<td>118 ± 13&lt;sup&gt;A&lt;/sup&gt;</td>
<td>12947 ± 2776&lt;sup&gt;B&lt;/sup&gt;</td>
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<tr>
<td>sFlk1-Fc (nonpregnant)</td>
<td>4</td>
<td>137 ± 2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>2269 ± 669&lt;sup&gt;B&lt;/sup&gt;</td>
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</table>

Pregnant and nonpregnant rats were administered adenovirus expressing Fc (control), sFlt1, or sFlk1-Fc protein. Mean arterial blood pressure (diastolic plus one third of the pulse pressure in mmHg) ± SEM and mean urine albumin/creatinine ratio (micrograms of albumin per milligram of creatinine) ± SEM were measured 8 days after adenoviral administration corresponding to the early third trimester in the pregnant rats. <sup>A</sup><i>P</i> < 0.05 and <sup>B</sup><i>P</i> < 0.01 as compared with the control group (Fc). Mean plasma sFlt1 levels were 388 ng/ml (pregnant) and 101 ng/ml (nonpregnant) in the sFlt1-treated rats. Mean plasma sFlk1 levels were 775 ng/ml (pregnant) and 1000 ng/ml (nonpregnant) in the sFlk1-Fc-treated rats.
Maynard: Determine if sFlt could be responsible for the clinical manifestations of preeclampsia

**Experiment 5:**
- Determine if sFlt-1 can produce glomerular endotheliosis.
- Took histologic specimens of rat kidneys of controls, controls treated with sFlk-Fc.
- Those treated with sFlt-1 showed glomerular endotheliosis.
Soluble Endoglin (Eng)

- Antiangiogenic protein that may inhibit TGF-B1 signals in the vasculature

- Prevents binding of TGF-B1 to its receptors and downstream signaling, including eNOS
Venkatesha et al: Purpose was to determine if soluble endoglin could be responsible for the clinical manifestations of preeclampsia

**Experiment 1:**
- Determine if levels of soluble Eng are elevated in preelcamptic placentas vs. normal controls.
- Performed northern blot and western blot for endoglin on the placentas of preeclamptic and normal controls.
- Soluble eng if 4 times higher in preeclamptic patients.
Venkatesha et al: Purpose was to determine if soluble endoglin could be responsible for the clinical manifestations of preeclampsia

- **Experiment 2:**
  - Determine if the serum levels of soluble Eng are higher in preeclampsia than in normal controls.
  - Used ELISA to quantify the levels of soluble Eng in preeclampticics and controls
    - 3 times high concentration in individuals with mild preeclampsia
    - 5 times higher concentration in individuals with severe preeclampsia
    - 10 times higher in individuals with HELLP
Venkatesha et al: Purpose was to determine if soluble endoglin could be responsible for the clinical manifestations of preeclampsia

**Experiment 3:**
- Determine if soluble endoglin affects endothelial function.
- Looked at the effect of soluble endoglin of endothelial tube formation and found it negatively impacts it.
- Treated mice with adenovirus containing sFlt and sEng
  - Increased capillary permeability
  - Worse in the sFlt group than the sEng group
  - Additive with both sFlt and sEng.
Figure 3 sEng inhibits capillary formation and increases vascular permeability. (a) Angiogenesis assays were done using human umbilical vein endothelial cells (HUVECs) in growth factor-reduced Matrigel and performed in the presence of 1 μg of recombinant sEng, sFlt1 or both, and endothelial tube lengths quantified. A representative experiment ($n = 4$) is shown, with average tube lengths (in pixels) indicated below the panels. Tube length results were quantified, showing a 62.2% ± 3.9, 62% ± 4.1 and 39.7% ± 4.2 reduction in sEng, sFlt1 and sFlt1+sEng, respectively ($P < 0.01$ versus controls).

(b) Microvascular permeability assessed by Evans blue leakage in mice injected with adenovirus containing Fc (Control), sEng, sFlt1 or sFlt1+sEng. Leakage of Evans blue was quantified in the various organs. Data represent a mean of four independent experiments. *$P < 0.05$ compared to controls, # $P < 0.05$ compared to sFlt1 alone.
Venkatesha et al: Purpose was to determine if soluble endoglin could be responsible for the clinical manifestations of preeclampsia

**Experiment 4:**

- Determine if sFlt and sEng could cause clinical preeclampsia.
- Injected adenovirus with sFlt and sEng into pregnant rats.
- Collected clinical data at 17-18 days
  - sEng led to an increase in MAP and proteinuria.
  - More pronounced in rats injected with sFlt
  - Worst with rats injected with both sEng and sFlt.
Experiment 5:
- Determine if sEng blocked the effects of TGF-B
- Isolated rat renal microvessels, applied TGF-B1 and B3 and found vasodilation, which was attenuated by sEng.
- VEGF and TGF-B had additive effects of vasodilation, which were blocked with sFlt and sEng in concentrations found in individuals with preeclampsia.
Systemic Maternal Endothelial Dysfunction
Main Clinical Manifestations

- HTN
- Proteinuria
- Reduced GFR
Hemodynamic Changes in Normal Pregnancy

- Increase in cardiac output
- Expanded circulatory volume
- Decrease in peripheral vascular resistance
- Overall minimal change in systolic pressure but more pronounced change in diastolic pressure
Hemodynamic Changes in Preeclampsia

- Decreased circulating volume
- Edema
- HTN
sFlt and soluble Eng prevent vasodilation normally seen in pregnancy

Increased numbers of vasoconstrictors:
- Increase in vasoconstrictors: endothelin, thromboxane A2, over dilators: NO and prostacyclin.
- Asymmetric dimethyl arginine, which inhibits nitric oxide synthase, is higher in patients with pre-eclampsia

Increased sensitivity to RAS:
- During pregnancy the RAS is upregulated, but there is resistance to its pressor effects.
- One reason may be the high levels of Ang(1-7), which inhibits angiotensin II.
- In pre-eclampsia, the levels of ang(1-7) are lower.
- IgG antibody to AT-1 has also been found in patients with pre-eclampsia.
Proteinuria

“glomerular endotheliosis”
- enlarged glomerular volume
- swelling of endothelial cells
- occlusion of capillary lumens.

sFlt injected into mice produces glomerular endotheliosis and a decrease in podocyte nephrin.

Preeclamptic women show reduction in nephrin and synaptopodin.
Sugimoto et al: Determine if anti-mouse VEGF neutralizing antibodies and sFlt-1 can induce proteinuria

- Took CD1 mice and injected them with a single injection of anti-VEGF antibody or sFlt-1/Fc at 3.25 picomoles/L or 32.5 picomoles per Liter
- Collected urine at 0, 1, 3, 5, and 24 hours after the initial injection
- Sacrificed mice and stained kidneys with antibodies to nephrin, podocin, CD-2Ap or alpha actinin 4
- Decrease in nephrin
A

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>VEGF Ab</th>
<th>VEGF Ab +VEGF</th>
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<tr>
<td>Nephrin</td>
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<td>CD2AP</td>
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<td>Podocin</td>
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<td>α-Actinin-4</td>
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</table>
Human Experiment:

- Kidneys from autopsies of 7 women who died from preeclampsia and 2 pregnant women who died accidentally (MVAs)
- Light microscopy and immunohistochemical stains were performed
  - normal histology of the controls, glomerular endotheliosis in the 7 women with pre-eclampsia
  - decreased staining of synaptopodin and nephrin in the preeclamptic patients, no difference in podocin staining.
Animal Experiment:

- 5 mice were given either VEGF antibodies or sFlt-1/Fc and their kidneys were then stained for nephrin, podocin, and synaptopodin.
- VEGF was then added back to the mice and they were restained for nephrin, podocin, and synaptopodin.
- Decrease in nephrin and synaptopodin in the mice that were given anti-VEGF or sFlt-1, which reversed when VEGF was added back.
(a) sFlt-1 injection

Control  sFlt-1  sFlt-1+VEGF

Nephrin

Synaptopodin

Podocin
(b) Anti-VEGF Ab  Control  Anti-VEGF Ab  Anti-VEGF+VEGF

Nephrin

Synaptopodin

Podocin
Decreased GFR

- Normal pregnancy increase in GFR secondary to vasodilation
- Preeclampsia has a decrease in GFR
Compared 13 normotensive pregnant white women to 10 preeclamptic women. 36-38 weeks all women submitted a 24 hour urine for protein, had a CBC, BMP, and urea taken, and were infused with dextran-40 (neutral molecule), inulin, and PAH. 1 hour later the subjects voided.
Moran et al: Determine the renal hemodynamic in normal pregnancy compared to preeclampsia

- Calculation of the RPF, the GFR, and the FF.
- Mathematical models were used to calculate Kf and pi Gc.
- Dextran molecules were separated by chromatography in the urine by size to determine size selectivity.
- Compared to normal pregnancy, preeclamptic did not show a rise in RPF, showed a 50% reduction in Kf, and a decrease in passage of 31-63 Angstrom dextrans.
- 5 months postpartum the experiment was repeated in normal and preeclamptic women, and there was no difference
Diagnosis

- Clinical:
  - Check for proteinuria, HTN

- Serologic:
  - Serum uric acid $>$ 5.5 to 6 mg/dl
  - Levels of sFlt
  - Levels of soluble endoglin
Levine et al: determine if serum levels of sFlt-1, PlGF, and VEGF can be used to predict the onset of preeclampsia

- Nested case control study which Matched 120 normotensive control pregnant women to 120 women who developed pre-eclampsia
- Drew sFlt-1, PlGF, and VEGF levels before enrollment in the trial, at 26-29 weeks, at 36 weeks, when pre-eclamptic women developed HTN and proteinuria, and at delivery.
- In controls the levels of sFlt-1 remained constant until 33-36 weeks, when they increased 145 pg/ml per week until delivery
Levine et al: determine if serum levels of sFlt-1, PlGF, and VEGF can be used to predict the onset of preeclampsia

- The PlGF concentration rose during the first two trimesters, peaked at 29-32 weeks, and decreased after.
- In preeclamptic the concentration of sFlt-1 began to increase at 21-24 weeks of gestation, with a steeper increase at 29-32 weeks.
- The level of PlGF levels were lower than controls at 13-16 weeks onward, which is earlier than sFLT-1 levels increased and may indicate an inability to produce PlGF.
- VEGF levels for both controls and preeclamptics were low throughout pregnancy.
- Concluded that increased levels of sFlt-1 and reduced levels of PlGF can predict pre-eclampsia.
Levine et. al: Determine if the soluble endoglin rises in association with preeclampsia

- Nested case control study which compared 4 groups of 120 women: women with gestational hypertension, normotensive women who delivered babies SGA, normotensive women who delivered normal weight babies, and women with preeclampsia

- ELISA to determine levels of soluble endoglin, sFlt, and PlGF at different time points during pregnancy.
Levine et. al: Determine if the soluble endoglin rises in association with preeclampsia

- Found that for women who delivered normal babies the levels of soluble endoglin were stable until 33-36 weeks, at which point it started to rise until delivery.
- Levels of soluble endoglin rose earlier in all three other groups of women, but had the highest rise for the women with preeclampsia.
- The sFlt:PlGF ratio only rose for women with preeclampsia
Treatment

- Delivery of the placenta
Prognosis

- 3 times increased risk of future kidney biopsy
- 4-5 times increased risk of later developing ESRD
Linked the data in the Medical Birth Registry of Norway with the data in the Norwegian Renal Registry.  
DM, kidney disease, essential hypertension, or rheumatic disease before the pregnancies were excluded.  
Among women pregnant one-two times, preeclampsia in the first pregnancy was associated with a RR of ESRD of 3.2, in the second pregnancy RR of 6.7, in both pregnancies RR of 6.4.
Among women who were pregnant up to 3 times, preeclampsia in 1 pregnancy was associated with a relative risk of 6.3, preeclampsia in 2 pregnancies was associated with a relative risk of 15.5.

Preeclamptic pregnancy that was low birth weight or a preeclamptic pregnancy that was small for gestation age added to the risk of eventually developing ESRD.

Reason for the association is unknown.