

Society for Pediatric Pathology
Annual Perinatal Symposium

Preeclampsia

Sunday, October 19, 2003, 8:00 am -12:30 pm
Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Needs assessment: Developmental biology of the placenta has been identified as a rapidly advancing area of importance for diagnostic perinatal pathology and was the subject of the 2002 Perinatal Symposium. Preeclampsia is a critically important disease of pregnancy; one of the major causes of fetal and maternal morbidity and mortality throughout the world. Many of the recent advances in understanding the developmental biology of the placenta directly pertain to its diagnosis and treatment. This seminar is intended to meet the needs of pediatric pathologists, general pathologists who deal with pediatric pathology, pathology residents, fellows in pediatric pathology, and clinicians in perinatal medicine. To address these needs the Society for Pediatric Pathology has secured leading experts in both clinical and basic science to deliver a multidisciplinary presentation on this complex topic.

Objectives: Participants will acquire a working understanding of:

- (1) Clinical heterogeneity in preeclampsia,
- (2) The role of oxidative stress and endothelial dysfunction in the pathogenesis of preeclampsia
- (3) (3) The anatomic lesions predisposing to the development of preeclampsia.

Accreditation Statement: The Society for Pediatric Pathology is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

CME Credit: Participants will be eligible for 4.5 h of Category I CME credit.

Disclosures: In accordance with ACCME guidelines, the faculty members for this course have indicated that they have no significant or other relationship with a commercial company, entity or service (which would be discussed in this educational program) to disclose. The SPP has also required that the speakers disclose any products that are not labeled for the use under discussion.

SYMPOSIUM AGENDA

8:00-8:10AM **Introduction and Welcome** : *Raymond W. Redline, MD*

8:10-9:00AM **Clinical Aspects of Preeclampsia**

Baha Sibai, MD, Professor and Chairman, Dept. Obstet Gynecol,
University of Cincinnati College of Medicine

9:00-9:50AM **Oxidative Stress and Preeclampsia**

Carl Hubel, PhD, Assistant Professor, Dept OB/ GYN and Reproductive
Sciences, University of Pittsburgh School of Medicine

10:00-10:20AM **Coffee Break**

10:20-11:10AM **Preeclampsia: A Syndrome of Endothelial Dysfunction**

S. Ananth Karumanchi, M.D., Assistant Professor of Medicine, Beth Israel
Deaconess Medical Center & Harvard Medical School

11:10-12:00PM **Pathologic Aspects of Preeclampsia**

Yee Khong, MD, Placenta Research Unit, Dept of Histopathology, Women's and
Children's Hospital, North Adelaide, Australia

12:00-12:30PM

Panel Discussion: Preeclampsia (*B Sibai, C Hubel, SA Karumanchi, Y Khong*)
Moderator: *R Redline*

12:30PM **Adjournment**

Diagnosis and Management of Gestational Hypertension-Preeclampsia and HELLP Syndrome

Baha M. Sibai, M.D., Professor and Chair, Department of Ob/Gyn, University of Cincinnati

Gestational Hypertension-Preeclampsia

Learning Objectives

1. To discuss recent concepts regarding diagnosis and management of mild hypertension-preeclampsia
2. To describe candidates for expectant management of severe preeclampsia
3. To discuss protocol for expectant management including maternal-fetal testing and indications for delivery

HELLP Syndrome

Learning Objectives

1. To describe controversy in definition and diagnosis
2. To present clinical presentation and differential diagnosis
3. To review management and complications
4. To discuss counseling and long-term prognosis

References:

1. Sibai BM. Diagnosis and management of gestational hypertension and preeclampsia. *Obstet Gynecol* 2003; 102:181-92.
2. Sibai BM, Caritis S, Hauth J. What we have learned about preeclampsia. *Semin Perinatol* 2003; 27:239-46.

Oxidative Stress, Antioxidants and Preeclampsia: What's The Connection?

Carl A. Hubel, Ph.D., Magee-Womens Research Institute and Dept. OB/GYN and Reproductive Sciences

I. Learning objectives

1. To understand the concept of oxidative stress and to gain an appreciation of the gaps in our understanding of its role in preeclampsia.
2. To identify some of the potential sources of oxidative stress in the placenta and maternal circulation.
3. To understand some ways in which vitamin C might enhance nitric oxide bioavailability.
4. To understand the rationale behind the planned/ongoing trials of vitamins C and E supplementation toward prevention of preeclampsia.

II. Introduction. The oxidative stress hypothesis

A. Two-stage model of preeclampsia

1. The complex, multisystemic manifestations of preeclampsia (**Figure 1**) were poorly explained until about 15 years ago when Jim Roberts, Robert Taylor, and colleagues formally proposed that widespread endothelial cell dysfunction is the common link. There is abundant morphological, functional, and biochemical evidence to support this hypothesis.
2. Increasing evidence also supports the hypothesis that excessive production of oxygen and nitrogen-based free radicals (“oxidative stress”) is involved in the pathophysiology of preeclampsia.
3. Oxidative stress can be defined as an imbalance between reactive oxygen species (ROS) (such as superoxide anion ($O_2^{\cdot-}$)) and antioxidants (such as vitamin C), favoring an overabundance of ROS (**Figure 2**).
4. Preeclampsia is currently thought to be a 2-stage disorder (**Figure 3**). According to this model, the first stage is reduced placental perfusion frequently secondary to abnormal implantation and development of the placental vasculature. The second stage is the maternal response to this condition, characterized by widespread inflammation and maternal endothelial cell dysfunction.
5. The linkage between the two stages is an area of intense investigation. Placental factors are not solely accountable for the maternal manifestations of preeclampsia. Intrauterine growth restriction and preterm birth are commonly associated with abnormalities in Stage 1 but without the occurrence of a maternal syndrome. There is accumulating evidence that maternal constitutional predisposition to cardiovascular disease, unmasked or accentuated during the stress of pregnancy, is a key component in development of preeclampsia. Although not discussed in this talk, data suggest that many of these same constitutional factors also predispose to cardiovascular disease later in life.

B. Hypothesized role of oxidative stress

1. Increasing evidence supports the hypothesis that excessive production of ROS plays a critical role in linking stages 1 and 2, resulting in the clinical manifestations of preeclampsia.
2. In this context, ROS may mediate a decrease in the bioavailability of vasodilator nitric oxide (NO).

- Small (resistance) arteries from women with normal pregnancy exhibit enhanced endothelium-mediated vasodilatory responses relative to nonpregnancy arteries. However, this pregnancy adaptation is absent or attenuated in preeclampsia.
- An NO-dependent component is involved in some, but not all, vascular beds.
- There does not appear to be a post-receptor defect as vessels relax to exogenous NO donors.
- The status of NO biosynthesis in preeclampsia is controversial. Most data are consistent with increased, not decreased, NO production. (*see Bilodeau J-F and Hubel CA, 2003*)
- In hypercholesterolemia, circulating metabolites of NO are increased but NO-dependent relaxation is inhibited.
- Possible explanation of this paradox: Inactivation of nitric oxide (NO) by ROS, especially superoxide ($O_2^{\cdot-}$), is an important mechanism of decreased NO bioavailability in several vascular disease models:
 - hypercholesterolemia
 - angiotensin II-induced hypertension
 - chronic myocardial infarction

3. This leads to a working hypothesis:

- *Decreased bioavailability of NO, in part due to oxidative destruction of NO by ROS, contributes to impaired vasodilatory response/increased contractility in women with preeclampsia.*
- *As postulated with other cardiovascular diseases, important links will exist between the renin-angiotensin system, vascular ROS production, vitamin C, and impaired NO-dependent vascular function.*

The talk will touch on some data supporting this hypothesis and ways in which vitamin C (ascorbate) might enhance NO bioavailability.

4. In developing this hypothesis, current findings will be reviewed concerning:

- Oxidative stress and the placenta, candidate mechanisms, and translation to the maternal circulation and
- Rationale for vitamins C and E supplementation toward prevention of preeclampsia.

5. Important caveats:

- Given the evanescence of ROS, it is difficult to determine whether oxidative stress is a cause or an effect of any human disease.
- ROS are essential for normal biological processes such as signal transduction. If antioxidant systems were 100% efficient, the result would be death from reductive stress. Without a clear, mechanism-based strategy, there are inherent dangers in proposing use of highly specific antioxidants such as mimetics of superoxide dismutase (SOD) to cure complex diseases. For example, when cytotrophoblast cells are transfected to overexpress SOD they fail to undergo the normal fusion to multinucleated syncytiotrophoblast.

III. Oxidative stress and the placenta.

A. Maternal vascular supply to the placenta

1. In normal pregnancy trophoblastic invasion causes the diameter of the spiral arteries feeding the intervillous space to increase greatly and these arteries lose their smooth muscle and thus their

contractility. In preeclampsia and in fetal growth restriction (without preeclampsia) the physiological changes are greatly restricted, being limited to the superficial region of the endometrium or absent altogether (**Figures 4 and 5**).

2. Defective remodeling of spiral arteries in preeclampsia (and IUGR) likely results in reduced uteroplacental perfusion and focal regions of hypoxia.

3. Vasoconstriction at the level of the radial arteries and proximal segments of spiral arteries may contribute to intermittent perfusion of the intervillous space in normal pregnancy. Also uterine blood flow is reduced with uterine contractions and is altered by posture and activity. The incomplete remodeling of spiral arteries in preeclampsia (retention of smooth muscle) may cause blood flow to become even more variable/sporadic.

4. The resulting larger fluctuations of intervillous oxygen concentrations might result in a ischemia-reperfusion scenario with overproduction of ROS ($O_2^{\cdot-}$, H_2O_2 , $ONOO^-$). (See Burton and Hung, 2003)

B. Data consistent with hypoxia/reoxygenation effects in the preeclampsia placenta:

1. One of the major sources of ROS in posthypoxic reperfusion injury is xanthine oxidase. The dehydrogenase (type D) form requires NAD and produces uric acid and NADH. During hypoxia/ischemia, this form is converted to the oxidase (type O) form which requires oxygen and produces uric acid and $O_2^{\cdot-}$ during reoxygenation (**Figure 6**).

2. We found increased holoenzyme immunohistochemical staining in invasive trophoblast and placental villi from preeclamptic pregnancies. Placental bed site curettings (which contain cytotrophoblast) from women with preeclampsia showed increased holoenzyme and increased type O activity compared to normal pregnant (**Figure 7**).

3. Generation of ROS in preeclampsia might be facilitated by decreases in superoxide dismutase (SOD). SOD expression in invasive cytotrophoblasts is markedly decreased in preeclampsia.

4. Superoxide is known to inactivate NO in a chemical reaction forming the potent oxidant peroxy nitrite anion ($ONOO^-$). The reaction of NO with $O_2^{\cdot-}$ is 3 times faster than the rate of reaction of $O_2^{\cdot-}$ with SOD. Formation of $ONOO^-$ is thus favored when NO outcompetes SOD for $O_2^{\cdot-}$. Peroxynitrite can cause inflammation and nitration of tyrosine residues on proteins (nitrotyrosine). Nitrotyrosine thus provides a “footprint” of NO destruction and peroxynitrite-mediated damage (**Figure 8**).

5. As first reported by Les Myatt, Univ. Cincinnati, trophoblast nitrotyrosine is increased in preeclampsia, with particularly intense immunostaining in association with fetal blood vessels of floating villi (**Figure 9**).

6. Graham Burton et al. subjected villous samples from Caesarean deliveries to hypoxia-reoxygenation in vitro. They found greatly increased formation of nitrotyrosine residues and lipid peroxidation products (hydroxynonenal), closely mimicking that found in preeclampsia (**Figure 10**).

C. Other evidence of placental oxidative stress in preeclampsia (see also Hubel, 1999)

1. Increased ROS production (EPR spectroscopy)
2. Increased protein carbonyls, protein products of free radical exposure, in the placenta.
3. Increased lipid peroxides in decidua basalis

These findings suggest that oxidative stress is increased in the preeclampsia placenta. However direct evidence in vivo and evidence of causality remains sparse.

IV. ROS in the maternal circulation

- A. Activation of maternal leukocytes occurs during uteroplacental passage in preeclampsia
- B. Increased monocyte and granulocyte ROS production occurs with normal pregnancy and is further increased in preeclampsia
- C. Endothelium of maternal subcutaneous arteries of women with preeclampsia shows increased nitrotyrosine (73% vs. 3%), and decreased SOD, consistent with oxidative destruction of NO before NO reaches its intended targets.

V. Potential links between placental and maternal oxidative stress.

A. NAD(P)H oxidase (Figure 11).

1. The membrane-bound endothelial and vascular smooth muscle NAD(P)H oxidases are the major source of $O_2^{\cdot -}$ and H_2O_2 in vascular tissues. Increased activity of this enzyme is strongly associated with impaired NO-mediated endothelial function in vivo and with risk factors for atherosclerosis. NAD(P)H oxidases present in the placenta are massively stimulated in preeclampsia. (see Dechend et al *Circulation* 2003;107:1632-1639).

2) A major mechanism of NAD(P)H oxidase activation is through the angiotensin II AT1 receptor. Women with preeclampsia have agonistic autoantibodies directed against the angiotensin AT-1 receptor that increase ROS production by NAD(P)H oxidase in placenta and vascular smooth muscle (see Dechend et al *Circulation* 2003;107:1632-1639).

3) A chain of events can be envisaged in placenta and extending to maternal vasculature in women with preeclampsia:

AT1 autoantibody (and/or other factors) -> AT1 receptor stimulation -> NAD(P)H oxidase activation -> ROS production -> NO depletion and inflammation -> preeclampsia.

B. Preeclampsia plasma/serum has adverse effects on function of isolated arteries-- is the AT1 receptor involved?

1. Exposure of isolated arteries from humans or rodents to low concentrations of plasma from women with preeclampsia alters the behavior of these arteries such that they resemble arteries from preeclamptic women (increased contractile sensitivity and decreased NO-dependent relaxation). To further characterize the circulating factors we have used a bioassay system using isolated mesenteric resistance arteries from nonpregnant mice.

2.. In this bioassay system,

- Exposure of isolated arteries to low concentrations of preeclampsia plasma (1% v/v) dramatically decreased endothelial (NO)-mediated relaxation to methacholine (29% vs 84%) (Figure 12).
- Myogenic tone of arteries exposed to preeclampsia plasma was increased while tone after exposure to normal pregnancy plasma was decreased.
- The preeclampsia plasma-induced effects are reversed by the AT1 receptor antagonist, losartan (Figure 13).
- AT-2 Receptor Antagonist (PD123319) has no effect
- The adverse preeclampsia plasma effects are reversed after removal of the plasma IgG fraction suggesting antibody involvement.
- Preeclampsia plasma increases vascular reactive oxygen species generation.

3) These data suggest that an IgG antibody interacting with the AT1 receptor is responsible for the observed adverse effects of preeclampsia plasma on NO-mediated relaxation. Further work will determine the role of the autoantibody reported by Dechend et al. and whether oxidative destruction of

NO is involved in this model.

C. Vitamin C (ascorbate) is important for NO homeostasis (**Figures 14 and 15**).

1. Ascorbate is the “first line” water-soluble antioxidant.
2. Ascorbate radical, formed from quenching of more powerful oxidants, is unreactive.
3. Vitamin C regenerates vitamin E (synergistic action).
4. Intracellular ascorbate (mM) competes effectively with NO[•] for O₂^{•-}, limiting ONOO⁻ formation.
5. Plasma ascorbate (30-60 μM) inhibits membrane lipid peroxidation and formation of oxidized low density lipoprotein (oxLDL).
6. Ascorbate is important for preservation of BH₄, an important cofactor of nitric oxide synthase (NOS)
7. Ascorbate is necessary for release of NO from S-nitrosothiols. S-nitrosothiols, particularly S-nitrosoalbumin, constitute an important circulating reservoir of releasable NO. Effective release of NO from albumin requires that adequate amounts of vitamin C exist in the circulation.
8. S-nitrosoalbumin concentrations are increased in plasma of women with preeclampsia (**Figure 16**). New data reveals that the effective concentration of ascorbate influencing NO release is in the physiologic plasma range. Release of NO is inhibited by the decreases in plasma ascorbate observed in women with preeclampsia. (**Figure 17**).
9. Several aspects point to the relative safety of vitamins C and E.

D. Ascorbate is decreased in the maternal circulation in women with preeclampsia.

Is uteroplacental ischemia the culprit?

1. Vitamin C concentrations are decreased in preeclampsia as first shown in 1964.
2. A beneficial role for antioxidants has been supported by a recent study of women at high risk of preeclampsia in which oral administration of 1 gram of vitamin C and 400 IU vitamin E daily from 22 weeks gestation was associated with a reduction in markers of endothelial dysfunction and a decrease in incidence of preeclampsia (*see Chappell et al. 2002*) (**Figures 18 and 19**).
3. A followup study demonstrated that antioxidant administration was associated with improvement in biochemical indices of the disease relating to oxidative stress and placental function.
4. What might cause the deficit in plasma ascorbate reserves in preeclampsia?
 - Increased risk may be associated with decreased dietary intake (Zhang C. et al. 2002).
 - Evaluation of women in the placebo arm of the study revealed that, relative to a low risk group, high risk women with abnormal uterine artery doppler evidenced lower ascorbate levels throughout pregnancy along with increased lipid peroxidation products. This was seen in women who subsequently developed preeclampsia and in women who delivered of small for gestational age babies. This suggests that placental ischemia leads to lower ascorbate and increased oxidative stress in the maternal circulation (*see Chappell LC et al. 2002*) (**Figure 20**).
5. Support for the concept that subnormal ascorbate leads to decreased NO-dependent function in the maternal circulation: Using a special strain of rats that (like humans) cannot synthesize vitamin C and thus are dependent upon dietary sources of the vitamin, we have shown that Moderate deprivation of dietary ascorbate leads to
 - Increased superoxide in mesenteric arteries
 - Increased lipid peroxidation
 - Decreases in mesenteric artery NO-dependent vasodilator function, but only in the setting of pregnancy

- IUGR

6. The concept that reduced placental perfusion leads to maternal oxidative stress is supported by additional experiments using these dietary vitamin C-dependent rats.

- Reduced uteroplacental perfusion (by abdominal aorta and utero-ovarian artery clip) results in decreased maternal ascorbate in pregnant rats incapable of synthesizing ascorbate (but less so in rats capable of synthesizing ascorbate).

VI. Maternal contributions to oxidative stress

A.. Risk factors in common For atherosclerosis and preeclampsia (**Figure 21**)

- Hypertension
- Diabetes
- Collagen vascular disease
- Increased plasma homocysteine
- Obesity
- Insulin resistance syndrome

B.. The Atherogenic Lipoprotein Phenotype in women with preeclampsia (**Figure 22**)

- Increased plasma triglycerides (TG-rich lipoproteins)
- Predominance of small, dense LDL particles
- Decreased HDL-cholesterol
- Post-prandial lipemia
- Increased free fatty acids

I. Underlying factors: insulin resistance and increased human placental lactogen

C. One result of increased free fatty acids in the circulation: Free fatty acid-mediated conformational changes in albumin increase the redox activity of albumin-associated Cu, converting albumin from an antioxidant to a prooxidant. This results in oxidative depletion of plasma ascorbate (**Figure 23**).

D. Interestingly, one of the signals increasing the release of free fatty acids from maternal adipocytes is human placental lactogen. This is an example of cross-talk between placental and maternal pathways leading to oxidative stress.

VII. Summary

Oxidative stress, due to both placental and maternal factors, may contribute to the pathophysiology of preeclampsia. Several observations suggest that decreased bioavailability of endothelium-derived NO, partly due to oxidative destruction of NO by ROS, might contribute to the vascular dysfunction and multisystemic pathology of preeclampsia, a phenomenon in which antioxidant vitamins may play a beneficial role (**Figure 24**). Clear evidence of causality, however, is still lacking. In this regard, antioxidant clinical trials will be important next step.

VIII. References

1. Bilodeau J-F and Hubel CA. Current concepts in the use of antioxidants for the treatment of preeclampsia. J Obstet Gynaecol Can 2003; 25(9):742-50.
2. Hubel CA Oxidative stress in the pathogenesis of preeclampsia Proc Soc Exp Biol Med 1999;222:222-235.

3. Many A, Hubel CA, Fisher SJ, Roberts JM, Zhou Y. Invasive cytotrophoblasts manifest evidence of oxidative stress in preeclampsia. *Am J Pathol* 2000; 156:321-331.
4. Burton GJ and Hung T-H Hypoxia-reoxygenation; A potential source of placental oxidative stress in normal pregnancy and preeclampsia. *Fetal Maternal Medicine Rev.* 2003;14:97-117.
5. Chappell LC et al. A longitudinal study of biochemical variables in women at risk of preeclampsia. *Am J Obstet Gynecol* 2002; 187:127-136.
6. Dechend R et al. AT1 receptor agonistic antibodies from preeclamptic patients stimulate NADPH oxidase. *Circulation* 2003;107:1632-1639.

PREECLAMPSIA: A SYNDROME OF ENDOTHELIAL DYSFUNCTION

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Abbreviations:

PE-preeclampsia, VEGF- vascular endothelial growth factor, PlGF- placental growth factor, sFlt-1-soluble fms-like tyrosine kinase-1, HTN-hypertension, GN-glomerulonephritis

Learning Objectives

Our current understanding of the mechanisms of preeclampsia will be discussed including recent evidence suggesting that interference with the action of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) may play a central role in the pathogenesis. Briefly, the learning objectives are as follows:

1. To understand the clinical manifestations of preeclampsia and their pathophysiologic underpinnings: Evidence that preeclampsia is an endothelial disease.
2. To learn the central role of the placenta in the pathogenesis of preeclampsia: Defective placental vasculogenesis and differentiation along with placental hypoxia leading to secretion of circulating factors that cause the maternal syndrome
3. To understand the role of endothelial growth factors in the pathogenesis of preeclampsia: Evidence that excess sFlt-1 (endogenous anti-angiogenic protein of placental origin) may play a role in preeclampsia by decreasing the availability of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) and thus affect maternal endothelial health.
4. Implications for Diagnosis, Prevention, and Treatment: sFlt-1 may serve as a diagnostic marker for the early diagnosis of preeclampsia. Treatments aimed at shifting the balance in favor of pro-angiogenesis and endothelial health holds promise for this syndrome.

Outline of the talk

I. Clinical manifestations of preeclampsia (PE) and their pathophysiologic underpinnings.

Unified by endothelial cell activation and/or damage leading to hypertension, proteinuria, and edema suggesting that preeclampsia may be an endothelial cell disorder (1, 2).

1. All these clinical manifestations have at their root endothelial cell dysfunction or its consequences (i.e., end-organ hypoperfusion)
2. *In vitro* effects of PE serum on endothelial cell culture
Induces expression of cellular fibronectin, tissue factor, vWF, and thrombomodulin and endothelial prostacyclin production
3. Increased circulating concentrations of cellular fibronectin, thrombomodulin and vWF
4. *In vitro* effects of PE serum on intact vessels: impaired endothelium-dependent relaxation in myometrial vessels and *in vivo* impaired flow mediated vasodilation
5. Glomerular endotheliosis – pathological lesion of preeclampsia is characterized by glomerular endothelial cell swelling

There may be a factor in preeclamptic serum that affects maternal endothelial cells. To identify this putative factor, we must look to its probable source, the placenta.

II. The central role of the placenta

1. Clinical observations: PE only occurs in presence of placenta and resolution begins with removal of placenta
 - i. Extrauterine pregnancy: delivery of fetus (without placenta) leads to persistent preeclamptic signs/symptoms postpartum
 - ii. Increased risk in conditions with increased trophoblast mass, such as hydatidiform mole, multiple gestations
2. Animal Models
 - i. Injection of PE placental extracts into beagles causes a PE-like syndrome
 - ii. Constriction of uterine arteries in dogs and baboons causes hypertension and proteinuria. Aortic constriction in rhesus monkeys causes PE-like syndrome
3. Defective placental vascular development (3)
 - i. Brief review of normal placental vascular development:
Invasion of maternal spiral arteries by cytotrophoblast cells and transformation of maternal spiral arteries into dilated vessels. Resemblance of normal trophoblast invasion to the angiogenesis and vascular mimicry of malignancy
 - ii. In PE, there is defective placental development:
Small caliber maternal spiral arteries with shallow invasion by cytotrophoblasts
Failure of pseudovasculogenesis: Failure of cytotrophoblasts to convert from epithelial to endothelial phenotype (4)

Hypothesis: In preeclampsia, the diseased placenta produces a circulating factor, which affects the maternal endothelium.

III. The quest for the circulating factor; evidence that excess sFlt-1 may play a role

1. Many candidates have been suggested but none so far have been shown to be etiologic: NK-B, TNF-a, inflammatory cytokines (1, 2)
2. Description of soluble fms-like tyrosine kinase-1 (sFlt-1) or sVEGF-R1
3. In preeclampsia, placental production of excess sFlt-1 shifts the angiogenic balance in the maternal circulation (5)
 - i. Placental sFlt-1, circulating sFlt-1 and amniotic fluid sFlt-1 is up-regulated in PE
 - ii. Although total VEGF levels vary, free VEGF and PlGF levels are decreased in PE
4. *In vitro*, VEGF blockade by sFlt-1 resembles preeclampsia (tube formation assay, microvascular reactivity experiments) (5)
5. sFlt-1 produces hypertension, proteinuria, and glomerular endotheliosis in rats (the fulfillment of Koch's postulates for preeclampsia) (5)
6. Other evidence of VEGF's role in protecting against hypertension and proteinuria

Importance of VEGF in glomerular healing/proteinuria

- i. VEGF enhances glomerular capillary repair in anti-thy1 GN
 - ii. VEGF blockade increases proteinuria and impairs glomerular endothelial healing in mesangioproliferative GN
 - iii. Glomerular podocyte-specific VEGF knock-out mice develop high-grade proteinuria and histological lesion of PE (6)
 - iv. Human anti-angiogenic oncology trials: VEGF neutralizing antibodies and VEGF receptor inhibitors cause proteinuria and HTN (7)
 - v. Smokers have a decreased incidence of PE, and nicotine is pro-angiogenic (?)
7. Speculation: Might sFlt-1 also play a role in disrupting early placental development?
- i. Early, marginal increase in sFlt-1 may impair pseudovasculogenesis and placental vascular development
 - ii. Once placental ischemia is established, sFlt-1 production increases further, causing not just local placental effects, but also systemic maternal effects

IV. Implications for Diagnosis, Prevention, and Treatment

1. sFlt-1 and/or free PIGF as early diagnostic marker for PE
2. Treatments aimed at shifting the balance in favor of proangiogenic state using VEGF or PIGF might allow delivery to be safely postponed.
3. Polymorphisms in the sFlt-1 gene/promoter in pts with PE may illuminate pathogenesis. PE is linked to higher cardiovascular morbidity and lower cancer incidence; could this be due to these polymorphisms affecting sFlt-1 protein production?
4. Other synergistic anti-endothelial factors may play a role in PE

V. REFERENCES

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The Pathology of the Placental Bed in Pre-eclampsia.

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Learning objectives:

1. Development of the normal blood supply to the fetus and placenta
2. Features of abnormal placentation in preeclampsia
3. Comparison of pathology of preeclampsia with placenta accreta
4. Effects of a previous pregnancy on placentation

II. The placental bed in normal pregnancy

Placentation is defined as the apposition of fetal and parental tissues for the purposes of physiological exchange. Fetal and maternal tissues are closely opposed at several sites, such as the syncytiotrophoblast-intervillous space, chorion laeve-decidua parietalis and the placental bed. Various terms have been used for that junctional zone between the placenta and the decidua; these include transition zone, detritus zone, chorion basalis, penetration zone, niemansland, boundary zone, junctional zone and placental bed. In using the term placental bed, Dixon and Robertson emphasised the crucial role of the blood supply to the developing placenta and fetus. In this area, namely the decidua deep to the basal plate and the subjacent myometrium, there is intimate juxtaposition of fetal and maternal tissues. This area also contains the origins of the spiral arteries and the final destination of the extravillous trophoblast.[1]

Ovarian and uterine arteries, lateral to the uterus, anastomose and send penetrating branches through the serosa to form the arcuate system. Radial arteries course towards the endometrium and continue as basal arteries, which supply the innermost myometrium and basal endometrium, and spiral arteries, which are hormonally-responsive and supply the rest of the endometrium.

Placentation in the human is characterised by excessive decidualisation of the endometrial stroma and invasion of the placental bed by extravillous trophoblast. Decidualisation of the endometrial stroma proceeds from the time of implantation and rapidly spreads through the entire endometrium.[2] Degenerative changes, such as necrosis and fibrin deposition, and infiltration of the decidua by endometrial granulocyte are seen.

During early pregnancy, extravillous trophoblast derived initially from the primitive cytotrophoblast shell and, later, the anchoring villi infiltrates into the decidua and superficial myometrium -- the placental bed. The extravillous trophoblastic cells extend into the placental bed by interstitial infiltration and in a retrograde manner in the spiral arteries. Thus, topographically, there are two subsets of extravillous trophoblast - the interstitial trophoblast and the endovascular trophoblast. The interstitial trophoblast tend to cluster around the spiral arteries which, prior to the arrival of the endovascular trophoblast, can be seen to be oedematous with early disintegration of the elastic tissue. From about six weeks gestation, endovascular trophoblast penetrates the spiral arteries migrating down the luminal

surface and plugging the lumen in areas. Migration extends from about six to 10 weeks gestation in the intra-decidual segments of the spiral arteries and, after a relatively quiescent period, from about 16 to 20 weeks gestation in the intra-myometrial segments.[3-5]

Electron microscopic studies have shown that the endovascular trophoblast becomes embedded within the spiral arterial walls and there is disorganisation of the internal elastic lamina and tissues of the media. Subsequently, the muscular and elastic tissues of the spiral arterial wall become replaced by fibrinoid material resulting in a distended artery. With increase in blood volume there is seen in the second half of pregnancy, these compliant vessels become dilated, allowing for a ten-fold increase in blood flow. The transformation of the small calibre PAS-negative muscular spiral arteries to distended large calibre PAS-positive utero-placental arteries devoid of muscular and elastic tissues constitute the physiological vascular changes.

III. The maternal blood supply in preeclampsia

Several complications of pregnancy are characterised by abnormal placentation, in particular defective development of the placental bed vasculature. The seminal observation that physiological vascular changes are absent in the intra-myometrial segments of the spiral arteries in pre-eclampsia has been confirmed by many investigators.[2] Furthermore, this defect of absence of physiological vascular changes extends to the intra-of decidual segments of the spiral arteries.[6] Other investigators also have confirmed this finding. Since this deficit extends to the openings of the spiral arteries into the intervillous space, absence of physiological vascular changes can be identified from sections of the basal plate of the delivered placenta.

The implication is that, early in pregnancy, the interaction between trophoblast in maternal tissues is compromised for whatever reason resulting in the lack of physiological vascular changes. Morphological studies point to a defect in interstitial and endovascular trophoblast migration. Analogous studies of placentation in a golden hamster model suggest that the second wave of endovascular trophoblast migration may be defective in pregnancies destined to result in pre-eclampsia. The finding of a similar deficit of physiological vascular changes in decidual segments as well as in first trimester miscarriages would suggest that the first wave of endovascular trophoblast migration might also be defective. Furthermore, it has been observed that interstitial trophoblast migration in miscarriages is reduced. Clustering of spiral arteries by interstitial trophoblast early in pregnancy results in early structural changes to the spiral arterial walls. Thus, it is likely that there may be a temporal window of opportunity when interstitial and endovascular trophoblast must migrate and interact with spiral arteries, outside which defective placentation will result.[1]

The mean field area percentage of trophoblast cells in the decidua and myometrium is reduced in pre-eclamptic compared with normotensive women; since this finding is from placental bed biopsies from the third trimester, it implies restricted invasion of trophoblastic cells in pre-eclampsia earlier in pregnancy.[7]

Other pathological features of pre-eclampsia are acute atherosclerosis and persistent intraluminal endovascular trophoblast. Acute atherosclerosis is characterised by fibrinoid necrosis of the vessel wall with a perivascular mononuclear cell infiltrate and lipid-laden macrophages. Acute atherosclerosis labels for lipoprotein-a, which is thrombogenic and atherogenic, and it is not surprising therefore that thrombosis is often seen within these uteroplacental arteries.[8] It has been suggested that acute atherosclerosis, bearing a marked similarity to the vessels seen in allograft rejections, represent a manifestation of aberrant maternal fetal

interactions. Acute atherosclerosis is seen in arteries that have not undergone physiological changes and, accordingly, can be seen in the arteries in the decidua parietalis in the extraplacental membranes.

Two gross abnormalities commonly seen in pre-eclamptic pregnancies result from these vascular abnormalities. The reduction in luminal diameter caused by the absence of physiological vascular changes, vasospasm, persistent intraluminal endovascular trophoblast, acute atherosclerosis and thrombosis can lead to a markedly impaired blood flow through the spiral arteries. This results in placental infarction. Placental abruption, manifest as retroplacental haematoma formation, is frequently seen in pre-eclamptic placentas. The source of the bleeding is unclear but occasionally a mass of vessels, some showing acute atherosclerosis or aneurysm formation, can be identified.

IV. Comparison of pathology of placenta accreta with pre-eclampsia

If pre-eclampsia is characterised by superficial implantation, then placenta accreta is its antithesis. The placenta is embedded directly onto myometrium in the absence of decidua. Whether this decidual deficiency is primary or secondary is unclear. The interstitial trophoblast in placenta accreta is hypertrophic and the majority is mononuclear, in contrast to the multinuclear placental bed giant cells seen in the third trimester in normal pregnancy or pre-eclampsia. As a consequence of the deep implantation, physiological vascular changes can be seen in the radial and, occasionally, the arcuate arteries. It is not surprising, therefore, that torrential haemorrhage can occur after the placenta or part of it is removed from the myometrial adherence.

It is curious that there is a trend to increased sex ratio in pre-eclampsia (1.24 in primigravid and 1.09 in multigravid women) but in women with a retained placenta, the sex ratio is inverted (0.88) [reference population 1.07]. The contrast between the decidua-rich, trophoblast-poor, superficially implanted placenta in pre-eclampsia and the decidua-poor, trophoblast-rich, deeply implanted placenta in the placenta accreta would suggest that these two conditions are diametrically opposed. Nevertheless, there is a small group of women in whom there is placenta accreta and who suffer from pre-eclampsia.[9]

Effect of a pregnancy on future placentation.

One of the remarkable features of pregnancy is that those uteroplacental arteries, devoid of muscular and elastic tissue, must be reconstituted following parturition. Failure to do so can result in secondary postpartum haemorrhage as demonstrated by subinvolution of the placental bed in curettage specimens. In this situation, there is persistence of the fibrinoid wall, and occasionally trophoblastic cells, and a patent arterial lumen that readily allows haemorrhage through these vessels there are devoid of muscle and elastic tissue capable of contracting.

Although muscle and elastic tissue are reconstituted within the spiral arteries postpartum, the structural changes are not completely resolved. Image analysis showed that duplication and fragmentation of the internal elastic lamina increased with increasing parity with differences between nulliparity and parity 1, 2, 3, or 4 but not between other parity pairings. The proportion of non-muscular tissue within the spiral arterial wall also increased with increasing parity. These permanent anatomical changes in the spiral arteries can modify subsequent vascular remodelling in a subsequent pregnancy by facilitating easier penetration of endovascular trophoblast. This provides an anatomical basis for the higher incidence of pre-eclampsia in nulliparous women and the reduced risk in multiparous women.[10]

V. Summary

Vascular lesions in the placental bed in pre-eclampsia are a lack of physiological vascular changes, persistence of intraluminal endovascular trophoblast and acute atherosclerosis and thrombosis. These have the effect of reducing blood flow through the arteries supplying the placenta. Pregnancy-induced vascular changes are not completely resolved following pregnancy and are a likely explanation for the reduced risk of pre-eclampsia in multiparous women.

VI. References

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