Published in final edited form as:

J Reprod Immunol. 2009 December ; 83(1-2): 134–138. doi:10.1016/j.jri.2009.08.006.

Novel approaches for mechanistic understanding and predicting preeclampsia

Satyan Kalkunte,

Department of Pediatrics, Women and Infants Hospital-Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

Zhongbin Lai,

Department of Pediatrics, Women and Infants Hospital-Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

Wendy E Norris,

Department of Pediatrics, Women and Infants Hospital-Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

Linda A Pietras,

Department of Pediatrics, Women and Infants Hospital-Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

Neetu Tewari,

Department of Pediatrics, Women and Infants Hospital-Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

Roland Boij,

Department of Pediatrics, Women and Infants Hospital-Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

Stefan Neubeck,

Placenta-Lab, Department of Obstetrics, Friedrich-Schiller-Universität, Jena, Germany

Udo R Markert, and

Placenta-Lab, Department of Obstetrics, Friedrich-Schiller-Universität, Jena, Germany

Surendra Sharma*

Department of Pediatrics, Women and Infants Hospital-Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

Abstract

Despite intense investigation, preeclampsia (PE) remains largely enigmatic. Relatively late onset of diagnostic signs and heterogeneous nature of the disease further contribute to poor understanding of its etiology and clinical management. There exist no concrete animal models that can provide mechanistic underpinnings for evaluating targeted therapeutic intervention. Poor cross-sectional findings with potential biochemical markers reported so far have proved counterintuitive and suggest

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*Corresponding author: Dr. Surendra Sharma, Department of Pediatrics, Women and Infants Hospital, 101 Dudley Street, Providence, RI 02905, USA. ssharma@wihri.org.

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a need for novel approaches to predict the early onset of disease. Because of the co-onset of local placental anomalies and systemic manifestation of symptoms, it is highly likely that serum from PE patients can provide a “blueprint” of causative factors. Proteomic and/or functional analysis of maternal serum are expected to predict the onset of disease ahead of manifestation of clinical symptoms. A serum-based predictive assay should overcome complexities resulting from the heterogeneous etiology of PE. This review attempts to address some of these issues and discuss the signature biochemical serum factors and propose new and better ways to predict PE.

Keywords

Preeclampsia; Pregnancy serum; Biological assay; Proteomics

1. Introduction

Mammalian reproduction involves a complex but highly choreographed sequence of molecular processes. These processes include interactions between the hormonally stimulated uterus and the developing blastocyst, implantation, a period of placental and fetal development, and a terminal pathway composed of activation of the decidual cells and fetal membranes, myometrial contractility, and cervical ripening (Matzuk et al., 2002; Paria et al., 2002; Abrams and Pickett, 1999). Although the hormonal milieu, metabolic changes, and placental microenvironment are programmed in a pregnancy compatible manner, pregnancy remains an immunological and hormonal paradox (Medawar, 1953). Perturbed pre-existing maternal health or altered local milieu during the course of pregnancy can lead to pregnancy complications such as preeclampsia (PE).

Hypertensive disorders of pregnancy have remained enigmatic and pose a major public health problem and affect approximately 5–10% of human pregnancies. PE is clinically associated with maternal symptoms of hypertension, proteinuria and glomeruloendotheliosis. PE is strictly a placental condition because of its clearance after placental delivery. It can be fatal in many cases and is a cause of morbidity and mortality in the mother, the fetus and the newborn. Pregnancy-associated hypertension is defined as blood pressure of $>140/90$ mm Hg on at least two occasions, 4–6 weeks apart after 20th week of gestation. Proteinuria is defined by excretion of 300 mg or more of protein every 24 h or 300 mg/L or more in two random urine samples taken at least 4–6 h apart (Report of the National High Blood Pressure Education Program, 2000). The fetal syndrome associated with PE entails growth restriction, reduced amniotic fluid and abnormal oxygenation (Sibai et al., 2005). However, the onset of the clinical signs and symptoms can result in either early or near-term PE without affecting the fetus or PE that is associated with low birth weight and preterm delivery (Vatten and Skjaerven, 2004; Dekker and Sibai, 2001). The heterogeneous pathogenesis of disease is further confounded by preexisting vascular disease, multifetal gestation, metabolic syndrome, obesity or previous incidence of PE. These observations suggest bidirectional maternal and fetal contribution. It is then imperative to predict onset of this syndrome early enough so that a therapeutic and palliative regimen can be undertaken.

2. Possible contributors of placental pathology in PE

The two stage hypothesis that proposed poorly perfused placenta (Stage I) causing release of factor(s) leading to maternal symptoms (Stage II) needs reevaluation. As observed in some cases of intrauterine growth restriction (IUGR) (Khong et al., 1986) and preterm birth (Kim et al., 2003), reduced perfusion of the placenta that occurs secondary to failed spiral artery transformation may not be sufficient to cause preeclampsia. This implies that the intrinsic maternal factors stemming from genetic, behavioral and physiological conditions contribute

to adaptability or lack of it that may lead to maternal syndrome. This may be particularly apparent in oxidative stress-induced release of “causative factors” from the poorly perfused placenta and their impact on maternal syndrome (Roberts and Hubel, 1999). Women with intrinsically poor “reduction potential” would then be prone to systemic manifestation of oxidative stress-mediated injury.

Moreover, it is now being recognized that maternal factors may contribute to programming of Stage I of PE. In this scenario, the adverse factors released by a presumed adaptive response in the placenta may modify maternal metabolism to increase nutrient availability. It is thus not surprising that a significant number of infants delivered by mothers with PE are not growth restricted. There are no clear markers that can differentiate Stage I from Stage II, or those that define PE with or without IUGR or other settings of abnormal placental perfusion. It is then possible that subtypes of PE exist based on multiple factors that link Stage I and Stage II, which thus may need individualized intervention.

Despite poor mechanistic understanding of placental pathology associated with PE, increasing evidence points to several critical anomalies common to this disease. A plethora of recent studies have suggested an increase in apoptosis in villous trophoblasts from PE/IUGR deliveries (Allaire et al., 2000; Levy et al., 2002; Heazell and Crocker, 2008). Although, the gestational period during which this process is triggered is unknown, the detection of several intrinsic and extrinsic apoptotic signaling molecules in the placental bed suggests its necessary role during placentation. Moreover, unlike normal pregnancy, villous placental explants from PE have an increased sensitivity and susceptibility to apoptosis on exposure to pro-inflammatory cytokines suggesting altered programming of the apoptotic cascade pathway (Crocker et al., 2004; Levy et al., 2002). It is possible that incomplete spiral artery transformation resulting in reduced placental perfusion (Stage 1) in PE leads to focal regions of hypoxia that, in turn, trigger the release of various vasoactive factors from the placenta causing widespread dysfunction of maternal vascular endothelial cells (Gilbert et al., 2008; Mutter and Karumanchi, 2008). *In vitro* studies have suggested that hypoxia can lead to apoptosis, increase in oxidative stress, shedding of villous microparticles, and elevated production of anti-angiogenic factors such as sFlt-1 (Redman and Sargent, 2000; Hung et al., 2002; Nevo et al., 2006).

Interestingly, in a rat model of reduced uterine perfusion, ligation of both the lower abdominal aorta and the ovarian arteries on gestational day 14 mimicked *in-utero* placental ischemia. These conditions induced only some of the PE-like symptoms, including elevated blood pressure, IUGR, enhanced soluble endoglin and HIF-1 α , and decreased hemeoxygenase-1 expression in the placenta suggesting a causal role of placental ischemia/hypoxia in PE (Gilbert et al., 2008). However, the model itself is based on invasive and traumatic procedures and the study does not show any effect on proteinuria and/or kidney pathology. It is well known that the uterine microenvironment experiences low oxygen tension at the time of implantation and during the first trimester. It is thus possible that deficiency in placental perfusion and extended low oxygen tension in the placenta result in increased cell death and necrosis. Some of these events may promote induction of cytotoxic factors, which when coupled with intrinsic defects such as IL-10 deficiency may lead to uncontrolled manifestation of inflammation and maternal symptoms of PE. Non-invasive *in-vivo* animal models that can study cumulative effects of multifactorial cascade would be an important advancement in our understanding of the disease.

Another pathway that may contribute to the etiology of PE is unscheduled and excessive activation of the complement cascade. This is highly likely to occur as a result of the maternal immune system responding to paternal antigens and inflammation. However, the placenta from normal pregnancy expresses complement regulatory proteins and may control activation of complement factors (Tedesco et al., 1993). Our understanding of the role of complement

activity in adverse pregnancy outcomes stems from work on anti-phospholipid syndrome and spontaneous fetal loss in the mouse models. In animal models, it has been shown that complement activation induces dysregulation of angiogenic factors and causes fetal rejection and growth restriction (Girardi et al., 2004; Girardi et al., 2006). Despite the positioning of complement inhibitory proteins for protective role, increasing evidence suggests the involvement of complement activation in the pathogenesis of PE (Haeger et al., 1992; Lynch et al., 2008). It is then possible to envision reduced placental expression of complement regulatory proteins in a subpopulation of PE placentae early during pregnancy, causing increased complement activity, local apoptosis and release of factors that cause maternal symptoms. Interestingly, recent *in vitro* studies suggest that hypoxia enhances placental deposition of membrane attack complex (MAC) and apoptosis in cultured trophoblasts (Rampersad et al., 2008). The upstream factors that trigger complement activation are not clear.

During the process of spiral artery remodeling, the invading cytotrophoblasts undergo phenotypic changes with loss of E-cadherin expression and acquisition of VE-cadherin, platelet-endothelial adhesion molecule-1, vascular endothelial adhesion molecule-1, $\alpha 4$, and $\alpha v\beta 3$ integrins (Zhau et al., 1997; Bulla et al., 2005). Our studies have shown that third trimester trophoblasts with poor expression of VEGF receptors and VEGF-C, and robust expression of E-cadherin and anti-angiogenic factors, fail to participate in an endovascular cross-talk between trophoblasts and endothelial cells (Kalkunte et al., 2008a). It is then possible that endothelial cells provide additional signaling cues to direct the invading trophoblasts towards spiral arteries at the maternal-fetal interface.

In this context, the presence of specialized uterine NK cells ($CD56^{bright}CD16^{-}CD3^{-}$ phenotype) at the maternal-fetal interface during active placental angiogenesis is intriguing. Several groundbreaking studies have implicated these uterine NK (uNK) cells in decidualization, invasion of the trophoblast, and production of angiogenic factors and chemokines. NK cell-deficient mice display abnormalities in decidual artery remodeling and trophoblast invasion, possibly due to lack of uNK cell-derived $IFN\gamma$ (Ashkar et al., 2000; Hanna et al., 2006). Although replete with cytotoxic machinery, uNK cells remain tolerant at the maternal-fetal interface. Our studies demonstrate that empowerment of uNK cells with angiogenic factors keeps them non-cytotoxic. VEGF-C, a pro-angiogenic factor produced by uNK cells, is an important immuno-vascular link responsible for their non-cytotoxic activity. This phenotype is critical to their pregnancy compatible immuno-vascular role during placentation and fetal development (Kalkunte et al., 2008b, 2009). Further, other studies suggest that in women, the killer immunoglobulin-like receptors (KIR) HLA-C haplotype combinations determine the optimal trophoblast-cell invasion and spiral artery remodeling by integrating signals to decidual uNK cells (Moffett and Hiby, 2007).

3. Biochemical anomalies reflected in serum

Although the symptoms of PE manifest clinically after the 20th week of pregnancy, this is believed to originate in a perturbed molecular cascade initiating during early pregnancy. Longitudinal biochemical, bio-functional and molecular monitoring of maternal serum, plasma, cell-free DNA, urine or micro particles during pregnancy can thus possibly mirror the state of events at the maternal-fetal interface. However, the heterogeneous nature that the disease presents has hampered the search for discovery of biomarkers applicable to the majority of the patient population. Major putative serum factors found to be perturbed in PE include angiogenic-anti-angiogenic factors (VEGF, placenta growth factor), soluble Flt-1, soluble endoglin, glycoprotein hormones like inhibin A and activin A, placental protein 13, complement factor Bb, and inflammatory cytokines (Levine et al., 2004; Lynch et al., 2008; Baumann et al., 2007; Jonsson et al., 2006; Mohaupt, 2007). Due to low sensitivity, high false

positive rates and poor cross-sectional outcomes, routine screening for such multiple markers is yet to be accepted (Gagon and Wilson, 2008).

4. Novel tools for predicting preeclampsia

During the last decade, several approaches have been utilized to identify biomarkers that can be used to predict PE. These efforts have been driven by advances in newer techniques. These approaches obviate a prior knowledge of the target markers, and instead aim to find differences in the biological components (e.g., protein expression profiles) from healthy and diseased individuals. Genomics, proteomics or metabolomic approaches have been targeted. Studies based on protein high-throughput mass spectrometry (MS)-based proteomics are examples of this type of approach.

4.1. Proteomic analysis

The discovery, identification, and validation of proteins associated with a particular disease state such as PE are complex and often require hundreds of samples for quantitative assessment. Recent proteomic advances have made it possible now to study multiple samples with trace amounts of proteins. Surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) is a novel approach to biomarker discovery that combines two powerful techniques: chromatography and mass spectrometry. The technique uses chips to retain proteins on a solid-phase chromatographic surface that are subsequently ionized and detected by SELDI-TOF MS. One of the key features of SELDI-TOF MS is its ability to provide a rapid protein expression profile from a variety of biological and clinical samples. This technique can also be used to study protein–protein, and protein–DNA interactions. SELDI-TOF MS is used for protein analytics when dealing with trace quantities present in the samples (Issaq et al., 2002). Using this method, complex protein samples react with SELDI chips embedded with any of a variety of surface matrices (e.g., cation and anion exchange, hydrophobic, metal-binding, or functionalized antibody or protein surfaces) to bind proteins according to their functional groups.

Multiple studies have now been reported for the use of proteomic approaches to compare serum samples from normal pregnancy and PE patients. Using such an approach, it has been demonstrated that clusterin, amyloid A, an acute phase protein, inter- α -trypsin inhibitor heavy chain H4, kininogen 1, fibrinogen γ , and α chains of haptoglobin alleles 1 and 2 are dysregulated in PE (Watanabe et al., 2004; Heitner et al., 2006). Investigation of these dysregulated proteins in a mouse model, with a focus on their ability to cause the clinical symptoms of PE, or the consequences of neutralizing their adverse effects, is essential to validate such findings. Moreover, we could adapt such an approach to delineate global changes in proteins in serum samples from longitudinal studies involving patients with normal pregnancy outcome and those who go on to develop PE. The causal link associating the protein with the disease symptoms can then be established and validated using biological approaches as outlined below.

4.2. In vitro and in vivo biological assays to predict preeclampsia

Shallow trophoblast invasion of spiral arteries is widely considered to be a hallmark feature of PE, IUGR, and preterm labor or preterm with premature rupture of membranes (PROM) (Naicker et al., 2003, Kim et al., 2003). Cross-talk between uNK cells, trophoblasts and endothelial cells is a key event in spiral artery remodeling. Recently using a dual cell *in vitro* model, we showed that the cellular events of endovascular remodeling can be restructured in response to pregnancy milieu represented by normal pregnancy serum (NPS). We showed that unlike third trimester trophoblasts, the first trimester extravillous trophoblasts fingerprint the endothelial cells in a capillary network in the presence of NPS (Kalkunte et al., 2008b). This unique behavior of first trimester trophoblasts in the presence of endothelial cells offers a

suitable biological assay to study cell-cell interaction as well decipher components in the serum samples from adverse pregnancy outcomes. Use of such biological tools in a longitudinal study would provide a more unbiased approach to predict PE-like disorder early during pregnancy. Since local and systemic features of PE are inter-dependent, we propose that serum from pregnancy can provide a “blueprint” for inducing PE pathology and to mimic clinical symptoms associated with PE in a suitable animal model (Fig. 1).

In this context, our published results suggest that pregnant mice with genetic IL-10 deficiency are highly sensitive to low doses of inflammatory triggers leading to fetal demise, premature delivery, and IUGR (Murphy et al., 2005, 2009; Thaxton et al., 2009). Using this sensitive mouse model, it is thus possible to investigate the PE-inducing role of serum samples and its reversal by factors that are dysregulated in PE serum or other pregnancy-associated hormones or cytokines.

5. Conclusions

Based on our observations, we propose that maternal serum taken in early pregnancy can be used to predict PE prior to the onset of clinical symptoms. Furthermore, novel experimental approaches involving the use of PE serum samples in *in vitro* and *in vivo* biological assays may help us to unravel the heterogeneous etiology of PE.

Acknowledgments

This work was supported in part by an NIH grant P20RR018728 and Rhode Island Research Alliance Collaborative Research Award. We thank the members of the Sharma laboratory for their critical reading of the manuscript.

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Figure 1. Predicting preeclampsia: *in vitro* and *in vivo* biological approaches

An *in vitro* assay involves a matrigel-based interaction of endothelial cells and first trimester trophoblasts to form an endovascular fingerprint in response to pregnancy serum. Use of proteomics can reveal differences at the protein level and use of human serum in a pregnant mouse model *in vivo* is expected to provide confirmation of the impending clinical symptoms of preeclampsia.