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Bioactive Factors in Uteroplacental and Systemic Circulation Link Placental Ischemia to Generalized Vascular Dysfunction in Hypertensive Pregnancy and Preeclampsia

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Abstract

Preeclampsia is a pregnancy-associated disorder characterized by hypertension, and could lead to maternal and fetal morbidity and mortality; however, the pathophysiological mechanisms involved are unclear. Predisposing demographic, genetic and environmental risk factors could cause localized abnormalities in uteroplacental cytoactive factors such as integrins, matrix metalloproteinases, cytokines and major histocompatibility complex molecules leading to decreased vascular remodeling, uteroplacental vasoconstriction, trophoblast cells apoptosis, and abnormal development of the placenta. Defective placentation and decreased trophoblast invasion of the myometrium cause reduction in uteroplacental perfusion pressure (RUPP) and placental ischemia/hypoxia, an important event in preeclampsia. RUPP could stimulate the release of circulating bioactive factors such as the anti-angiogenic factors soluble fms-like tyrosine kinase-1 and soluble endoglin that cause imbalance with the pro-angiogenic factors vascular endothelial growth factor and placental growth factor, or cause the release of inflammatory cytokines, reactive oxygen species, hypoxia-induced factor-1 and AT₁ angiotensin receptor agonistic autoantibodies. The circulating bioactive factors target endothelial cells causing generalized endotheliosis, endothelial dysfunction, decreased vasodilators such as nitric oxide and prostacyclin and increased vasoconstrictors such as endothelin-1 and thromboxane A₂, leading to increased vasoconstriction. The bioactive factors also stimulate the mechanisms of VSM contraction including Ca²⁺, protein kinase C, and Rho-kinase and induce extracellular matrix remodeling leading to further vasoconstriction and hypertension. While therapeutic options are currently limited, understanding the underlying mechanisms could help design new interventions for management of preeclampsia.

Keywords

endothelium; vascular smooth muscle; extracellular matrix; pregnancy; hypertension; preeclampsia

INTRODUCTION

Preeclampsia (PE) is a pregnancy-related disorder characterized by hypertension (HTN) and occasionally proteinuria and edema in late second or third trimester. PE affects 5 to 8% of pregnancies and imposes a significant burden on the health care system with high maternal and fetal morbidity, intrauterine growth restriction (IUGR) and preterm birth. Without timely intervention, PE is conducive of a more grievous clinical picture of maternal seizures and convulsions which could culminate into coma and death [1-3].

Despite the severe consequences of PE on maternal and fetal health, the pathophysiological mechanisms are unclear. Studies have suggested certain demographic, genetic, and environmental predisposing and risk factors. Clinical studies have shown changes in the levels of several bioactive factors in women with PE. The observation that PE remits after delivery has also pointed out the placenta as the main culprit in PE. Due to the difficulty to perform mechanistic studies in women, animal models of hypertension in pregnancy (HTN-Preg) have been developed. These experimental studies have delved deeper into the pathophysiological mechanisms of PE and suggested that reduction in uteroplacental perfusion pressure (RUPP) and consequent placental ischemia/hypoxia could cause the release of bioactive factors that cause generalized vascular dysfunction and HTN. Bioactive factors could also increase endothelial cell permeability, and cause renal glomerular endotheliosis leading to proteinuria, and cerebral endotheliosis leading to cerebral edema. These observations have raised interest in understanding how the potential predisposing and risk factors could lead to RUPP and placental ischemia, how the localized RUPP and uteroplacental ischemia are linked to generalized changes in the systemic circulation and target damage of different maternal organs, and how the release of bioactive factors from the placenta into the circulation could result in generalized vascular dysfunction and HTN.

In this review, we use reports from Pubmed and other scientific data bases, as well as data from our laboratory to provide mechanistic insights into the pathophysiology of PE. We will discuss how certain demographic, genetic and environmental factors could promote localized abnormalities in uteroplacental cytoactive factors such as integrins, matrix metalloproteinases (MMPs), inflammatory cytokines and cells leading to vasoconstriction of placental vessels, apoptosis of trophoblast cells, defective placentation and RUPP. We will discuss how RUPP and placental ischemia/hypoxia could cause imbalance in pro-angiogenic/anti-angiogenic factors and stimulate the release of other bioactive factors such as inflammatory cytokines, reactive oxygen species (ROS), and hypoxia-inducible factors (HIF), and how these factors cause systemic endotheliosis, endothelial dysfunction, decreased vasodilators such as nitric oxide (NO) and prostacyclin (PGI₂), increased vasoconstrictors such as endothelin-1 (ET-1) and thromboxane A₂ (TXA₂), and increased mechanisms of vascular smooth muscle (VSM) contraction such as Ca²⁺, protein kinase C and Rho-kinase leading to increased vasoconstriction and HTN (**Figure**). We will also discuss how the knowledge of the potential factors involved could lead to better approaches for management of PE.

Demographic Risk Factors in PE

Maternal demographic factors such as age, height, and ethnic background may influence the risk of PE. Extreme maternal age (<16 or >40 years old) may be a risk factor of PE [3]. A study from Finland showed greater incidence of PE among women with advanced maternal age (9.4%) than younger women (6.4%) [4]. Also, a study from India reported higher incidence of PE with advanced maternal age [5]. Women of short stature may have higher risk of severe PE, and infants of short statured women are at a higher risk of perinatal mortality [6]. Ethnic background may also influence the incidence rates of PE, with African-American women having the highest rate (5.2%), while Asian women have the lowest rate (3.5%). When combined, African-American and Hispanic women have higher rates of PE than Caucasian and Asian women [7]. Also, out of 4 million births recorded by the National Vital Statistics Report, more African-American women (5%) suffered from HTN-Preg than Hispanic women (2.9%) [8]. African-American women show more PE-related complications such as placental abruption and still-birth compared to European women. Low folate levels and high homocysteine levels may be related to the incidence of PE among African women in Zimbabwe [9]. With regard to paternal ethnicity, among Asian and African-American women, the incidence of PE is higher if paternal ethnicity is different from maternal ethnicity (4.6% and 5.8%, respectively) than if paternal and maternal ethnicities are the same (3.2% and 5.2%, respectively). However, among Native-American women the incidence of PE is greater if paternal ethnicity is the same (9.7%) than if it is different from maternal ethnicity (3.3%) [10].

Hereditary and Genetic RISK Factors in PE

Hereditary and genetic factors may contribute to the risk of PE (**Table 1**). Daughters of women with PE have 2-fold increased risk of developing PE [11]. Studies have identified gene locus on maternal chromosomes 1, 2p13, 2q, 3p, 4q, 9, 10q, 11q23–24, 12q, 15q, 18, 22q, 2p25 and 9p13 with potential relation to PE [12, 13]. Also, women with the T allele of vascular endothelial growth factor (VEGF) 936C/T have lower levels of VEGF and higher risk of PE than women with VEGF 936C/C [14]. Mutations in placental genes are associated with higher incidence of PE, and 31 out of 36 placental genes are downregulated in PE [15]. Mutations in mitochondrial genes of the placenta may cause incomplete reduction of O₂ in the electron transport chain and in turn increase production of ROS and subject the placenta and maternal vessels to oxidative stress [16]. Polymorphism of Fas and Fas ligand genes may contribute to PE. In normal pregnancy (Norm-Preg), activated T cells express Fas which interacts with the Fas ligand on trophoblasts and causes apoptosis of T cells. However, the expression of Fas 670 G variant reduces Fas expression on activated T cells leading to reduced T cell apoptosis and increased trophoblast destruction [17].

Fetal genes could also contribute to PE. The interaction between genotype (AA) of the inhibitory Natural Killer (NK) cell receptor KIR and homozygous genotype of fetal major histocompatibility complex (MHC) molecule HLA-C2 increases in PE, leading to inhibition of NK cells and decreased trophoblast invasion. PE does not avert in the presence of maternal HLA-C2, suggesting that fetal HLA-C2 on trophoblast cells differ from maternal HLA-C2. However, simultaneous activation of the activating isoform of KIR on NK cells can prevent the inhibition of NK cells and mediate proper trophoblast invasion [18].

Pregnant women with fetus carrying the +14/+14 HLA-G genotype or gene-to-gene interaction between fetal HLA-G and maternal KIR2DL4 in multigravid pregnancy may predispose women to PE [19, 20]. Endoplasmic reticulum aminopeptidase 2 (ERAP2) regulates blood pressure (BP) and cytokine production in Norm-Preg. The variants of the fetal ERAP2 gene rs2549782 and rs17408150 increase the risk of PE. Fetal minor allele (G) of rs2549782 is linked to higher risk of PE in African-American women. Mutated fetal ERAP2 gene also predisposes Australian/New Zealand and Norwegian women to PE [21]. Mice having pups with deficient or mutated cyclin-dependent kinase inhibitor *cdkn1c* (p57Kip2), a regulator of embryonic growth, develop PE-like condition and show changes in the placenta indicative of RUPP [12].

Paternal genes may also contribute to the risk of PE. Women with hydatid-form mole-pregnancies in which the fetus acquires all the paternal chromosomes [22], or who have partners born from a PE pregnancy have a higher risk of developing PE [22, 23]. The maternal genital tract mucosa develops tolerance to paternal antigens due to repeated exposure of sperms from a single partner, and this protective mechanism may be lost in women who frequently change partners [24]. Deposition of sperms in the female genital tract causes the seminal vesicle-derived transforming growth factor- β (TGF- β) to bind to the sperms and initiates an immune response necessary for implantation of the embryo. This immune response is less developed with shorter duration of sperm exposure, acquisition of sperms surgically and use of contraception, thus predisposing women to PE [25]. Also, mutations of HLA-G and Insulin-like growth factor-2 (IGF2) genes in men can induce PE in their partners. Cytomegalovirus is present in semen of 5-10% of men which could decrease the expression of α 1 β 1 integrin and HLA-G in trophoblasts and increase the risk of PE in women [24].

Environmental RISK Factors in PE

The maternal lifestyle, diet, obesity and metabolic factors could subject women to PE. The prevalence of obesity is increasing, and about 28% of women aged 20-39 years in the United States are obese [26]. Increased body mass index (BMI) or waist-to-hip ratio are indicators of obesity [27]. Compared with women with BMI of 21, the risk of PE doubles at BMI of 26, triples at BMI of 30, and BMI >32.2 is a major maternal risk factor of PE [28, 29]. African-American women have higher tendency to gain weight during pregnancy, while Asian women have the least tendency [30]. Waist-to-hip ratio is a better indicator of intra-abdominal fat accumulation because it is easy to measure, not influenced by height, indicates central weight distribution, and could predict the onset of PE up to 16 weeks of gestation [27, 31]. Gestational obesity increases insulin resistance [27], insulin-sensitivity regulator adipocyte fatty acid-binding protein 4 [32], plasma and vascular levels of C-reactive protein, TNF- α , IL-6 and IL-8 and leptin thus contributing to vascular changes [33], and decreases adiponectin, a hormone that suppresses adhesion molecules in ECs and cytokine production in women with normal BMI [34]. Women with family history of type 2 diabetes [35] and with pre-pregnancy diabetes have higher risk of PE (~12%) than women without diabetes (~3%). Also, the rate of PE with placental abruption or infarction is greater in women with pre-pregnancy diabetes (2.2%) than in women without diabetes (1.8%) [35-37].

Minerals and vitamins may also influence the risk of PE. During pregnancy, the maternal Ca^{2+} homeostatic mechanisms undergo changes in order to meet the increasing maternal and fetal Ca^{2+} demand [38]. Studies have shown low serum Ca^{2+} in women with PE [5, 39, 40]. Low Ca^{2+} levels may cause release of parathyroid hormone and renin, and lead to increased VSM Ca^{2+} , vasoconstriction and HTN [5]. In pregnant rats, low Ca^{2+} intake increases pressor effects of AngII [41] while high Ca^{2+} diet (1.7%–2.1%) reduces BP and VSM reactivity [42]. In pregnant ewes, restricted Ca^{2+} intake is associated with decreased uterine blood flow, increased BP and proteinuria [43]. Randomized controlled clinical trials have suggested that Ca^{2+} supplementation 375–2000 mg/day in a population with low Ca^{2+} intake is a safe, effective and inexpensive measure to reduce the risk of PE [44–46]. Compiling data from 12 clinical trials led to the conclusion that Ca^{2+} supplement may reduce the risk of HTN-Preg by 50%. The composite outcome maternal death or serious morbidity was also reduced. The effect was greatest in women at high risk of PE and those with low baseline Ca^{2+} intake [47]. Ca^{2+} supplement has also been shown to reduce the risk of PE and preterm birth particularly in nulliparous women [48]. Other studies were less supportive of beneficial effects of Ca^{2+} supplement in reducing the risk of PE. The Ca^{2+} for PE Prevention (CPEP) trial has shown no significant effect of 2000 mg/day Ca^{2+} supplement on BP or incidence of PE. Also, when the analysis was stratified according to patient's compliance, there was no effect on PE even among women who were most compliant with treatment [49]. Other studies did not observe a lower risk of PE with greater dietary intake of Ca^{2+} , vitamin C, D, or E, Mg^{2+} , folate or elongated omega-3 fatty acids [50]. Further evaluation of these observations by the FDA have led to the suggestion that it is unlikely that consuming Ca^{2+} supplement during pregnancy would reduce the risk of HTN-Preg or PE [51]. Similarly, a World Health Organization clinical trial of Ca^{2+} supplement among low Ca^{2+} intake pregnant women concluded that it did not prevent PE, although it did reduce PE severity, maternal morbidity and neonatal mortality [52]. The discrepancy in the effects of Ca^{2+} supplement among various studies may be related to the study design. Significant heterogeneity was observed within the studies, with less effect of Ca^{2+} supplement in the larger clinical trials [47]. The discrepancy may also be related to the subjects' age. For example, studies that included large number of pregnant teen-agers, whose demand for Ca^{2+} exceeds that of pregnant adults, have shown that Ca^{2+} supplementation results in a significant decrease in BP and 12.4% decline in risk of PE [46].

Serum Mg^{2+} may show a decrease or no change in PE [5, 39, 40]. Mg^{2+} regulates protein synthesis and membrane potential in nerves and muscles. Mg^{2+} is positively associated with birth weight [1], reduces the risk of IUGR and PE, causes VSM relaxation [5], dilates cerebral vessels and reduces cerebral ischemia [53]. Diets rich in K^{+} and fiber also reduce the risk of PE [54]. Studies have shown a decrease in Zn^{2+} and an increase in uric acid levels in women with PE [5, 39, 40]. Increased uric acid levels may increase ROS and TNF- α in PE [55].

Vitamin D or cholecalciferol is an important cofactor in Ca^{2+} absorption and metabolism. Cholecalciferol is formed in the skin during exposure to sunlight and UV irradiation and absorbed from ingested food, then converted in the liver to 25(OH) cholecalciferol or 25(OH)D, and further hydroxylated by 1 α -hydroxylase in the kidney to 1,25 dihydroxycholecalciferol or 1,25(OH) $_2$ D $_3$, the most active form of vitamin D. Adequate

levels of 25(OH)D (>80 nmol/L) maintain health of the bones, the cardiovascular system and BP. 1,25(OH)₂D₃ levels are elevated during pregnancy due to increased activity of renal 1 α -hydroxylase and placental production. Also, vitamin D receptors are found in the placenta [38]. In Norm-Preg, methylation of the promoter region of the CYP24A1 gene increases 1,25(OH)₂D₃ levels in the placenta. Also, under normal conditions, the expression of 1 α -hydroxylase, 24-hydroxylase, vitamin D binding protein and vitamin D receptor is increased in cultured trophoblast cells [38]. Vitamin D deficiency and altered Ca²⁺ metabolism have been implicated in abnormal placentation and PE [56]. The levels of 1,25-(OH)₂D₃ in the maternal and umbilical cord are lower in PE than Norm-Preg [57]. PE is more common in winter possibly due to low serum 25(OH)D₃ and placental 1,25(OH)₂D₃ levels leading to impaired placentation [58] and up-regulation of TNF- α , IL-6 and IL-1 β [59]. Vitamin D receptor null mice show increased plasma renin, plasma ngII, and HTN. Also, in wild-type mice, inhibition of 1,25(OH)₂D₃ synthesis leads to increased renin expression, and 1,25(OH)₂D₃ injection causes renin suppression, supporting a role of 1,25(OH)₂D₃ in the regulation of the renin-angiotensin system and BP [60].

Vitamins may influence oxidants/antioxidants balance and the risk of PE. Serum levels of vitamins C and E are decreased in PE, and vitamin supplementation may reduce the incidence of PE in women at high risk [61]. Vitamin C and E when given at a dose of 1000 mg/day and 400 IU/day respectively, at 16–20 weeks of gestation may lower the risk of PE [62]. Folic acid, a coenzyme in nucleic acids synthesis and cell growth, may play a role in the implantation and placental development and could reduce the risk of PE by improving EC function in placental and systemic vessels and by lowering plasma homocysteine level [63]. Initial studies have shown that women receiving multivitamins containing folic acid show increased serum folate, lower plasma homocysteine, and 63% reduction in the risk of PE compared to women with no vitamin supplementation [64]. Also, the large multicenter Folic Acid Clinical Trial (FACT) has been designed to recruit 3,656 high risk women and evaluate folic acid supplementation throughout pregnancy as a new prevention strategy for PE [65]. The results obtained from the FACT trial will help determine if 4.0 mg/day folic acid supplementation starting in early pregnancy (8 to 16 weeks) until delivery is effective in preventing PE and its associated adverse outcomes in women with increased risk of developing PE. Follow-up studies of the participants should also evaluate the long-term outcomes of folic acid supplementation during pregnancy in the mothers and their offspring.

Preexisting medical conditions including renal or cardiac disease, mental stress, previous neonatal macrosomia, history of reproductive tract surgery and antepartum hemorrhage, primiparity, and chronic respiratory conditions may also be associated with PE [3].

RUPP and Uteroplacental Ischemia in PE

The remittance of symptoms of PE after delivery has pointed to the placenta as a central culprit in the disease. Experimental studies suggest that RUPP and consequent placental ischemia/hypoxia could be an important mechanism in PE [2, 66, 67]. By the end of the second trimester in Norm-Preg, trophoblasts invade the maternal decidua up to one-third of the myometrium and substitute VSM and elastic tissue in the vessels with fibrinoid material, thus converting the spiral arteries into low-resistance high-capacity conduit arteries and

ensuring sufficient blood supply to the growing fetus [68]. In PE, apoptosis of trophoblast cells and poor endovascular trophoblast invasion diminish the spiral arteries remodeling and diameter [69-71]. Also, retention of smooth muscle in the spiral arteries increases the vessels contractility and vasoconstriction [72], further decreasing uteroplacental blood flow and leading to RUPP [71]. While several studies support the role of RUPP in PE, the factors initiating RUPP and the factors released in response to RUPP are the subject of extensive research.

Potential Factors Causing RUPP

The predisposing risk factors of PE are believed to promote localized abnormalities in the placenta including abnormal expression of integrins and MMPs leading to decreased ECM remodeling and trophoblast invasion and increased uteroplacental vasoconstriction, as well as abnormal expression of cytokines, NK cells, macrophages and MHC molecules leading to an inflammatory and immune response and apoptosis of trophoblast cells (**Figure**).

Integrins are heterodimeric glycoproteins with non-covalently formed α - and β - subunits [73]. Integrins are expressed on the trophoblasts lining the spiral arteries and mediate proper trophoblast invasion during Norm-Preg. Before invasion of the myometrium, trophoblasts express adhesion molecules characteristic of epithelial cells such as integrins $\alpha 6/\beta 4$ and $\alpha 6/\beta 1$ and E-cadherin. As trophoblasts invade the myometrium, the epithelial cell-like adhesion molecules are replaced with integrins $\alpha 1/\beta 1$ and $\alpha v/\beta 3$, a process called vascular mimicry or pseudovasculogenesis [70]. In PE, the expression of integrins is different from that in Norm-Preg. Also in hypoxia, expression of fibronectin and integrin $\alpha 5$ are high while expression of integrin $\alpha 1$ is low, supporting that decreased oxygenation of the spiral arteries could alter the synthesis of integrins and fibronectin in the placenta [74]. The interaction between integrins on leukocytes and the adhesion molecules ICAM-1, VCAM-1 and hs-CRP on ECs may also facilitate adhesion to ECs. In Norm-Preg, down-regulation of EC adhesion molecules minimizes leukocyte adhesion to ECs thus allows dilation and maintained blood flow in the spiral arteries. The plasma levels of sICAM-1, sVCAM-1 and hs-CRP are greater in PE than Norm-Preg women, and hs-CRP levels may predict the severity of PE [75]. Increased ICAM-1 has also been linked to increased EC $[Ca^{2+}]_i$ and platelet aggregation in PE [73].

MMPs are zinc-dependent degradative enzymes involved in tissue remodeling and other cellular functions. During pregnancy, MMPs such as MMP-2 and -9 remodel ECM to allow the spiral arteries to invade the myometrium. MMP-2 and -9 are up-regulated in the myometrium and aorta of Norm-Preg rats. In PE, genetic polymorphisms may modify transcription of MMP-2 and -9 genes [76]. MMP-2 degrades collagen I, II, III, IV, V, VII, X and XI while MMP-9 degrades collagen IV, V, VII, X and XIV. With low MMP levels, excessive collagen deposition may affect smooth muscle growth and decrease spiral arteries remodeling [77].

During Norm-Preg, decidualization, a process which converts uterine stromal cells into epithelial-like decidual tissues, causes considerable remodeling of the vessels. Decidualization correlates with the appearance of decidual Natural Killer (dNK) cells, the most abundant immune cells at the maternal-fetal interface constituting 50-70% of the host

defense cells. IL-15 may stimulate dNK cells to release interferon- γ (IFN- γ) which is important for remodeling of the spiral arteries, and IL-15 mRNA and protein levels may decrease in PE placenta [78]. Also in PE, reduced secretion of NK cell factors such as VEGF, angiopoietin-1, angiopoietin-2, placenta growth factor (PlGF) and cytokines may render the dNK cells unable to facilitate trophoblast development in the decidua [79]. Other studies link an increase in IL-15 levels, stimulation of dNK cells and release of IFN- γ to higher incidence of miscarriages and PE [80]. IFN- γ levels increase in plasma, leukocytes and decidua of women with PE [80] likely due to the presence of +874 T allele in the first intron of the IFN- γ gene [81]. Also, dNK cells release the pro-inflammatory cytokine IL-17 which may cause vascular changes and HTN in PE [82].

In PE, cytokines such as TNF- α modify the expression of adhesion molecules in placental vessels [83], and induce abnormal production of MMPs [76], apoptosis of trophoblasts and expression of plasminogen activator inhibitor-1 (PAI-1) thus reducing trophoblast invasion [84] and promoting placental ischemia. In the placental decidua, monocytes can differentiate into dendritic cells or macrophages. IL-6 shifts the differentiation of monocytes to macrophages, and the macrophages produce TNF- α which promotes apoptosis and reduces trophoblast invasion [84]. LIGHT, a TNF superfamily member, is also increased in PE and may contribute to placental damage and ischemia [85]. In Norm-Preg, T-helper 2 cells release IL-10, an anti-inflammatory cytokine that is abundant in the trophoblasts and helps develop maternal-fetal tolerance, regulates placental vasculature to ensure adequate blood supply to the fetus, and reduces abnormal expression of MMPs. IL-10 is decreased in PE possibly due to an IL-10-819 promoter polymorphism [86] or increased IL-10-082 G allele [81]. Also, hypoxic placental trophoblasts show decreased IL-10 and increased pro-inflammatory cytokines [87].

Major Histocompatibility Complex (MHC) molecules interact with the inhibitory NK cell receptors KIR, CD 94/NKGs and ILT to prevent maternal NK cells from killing placental and fetal tissues. In Norm-Preg, trophoblasts express high levels of the MHC-I molecules HLA-C, HLA-E and HLA-G, which interact, respectively, with the inhibitory KIR, CD 94/NKGs and ILT-2 receptors on NK cells [88]. Decreased HLA-C/KIR interaction due to absence of KIR despite increased expression of HLA-C2 increases the risk of PE [18]. However, increased HLA-C/KIR interaction in PE-induced mice showed excessive inhibition of NK cells and poor trophoblast invasion. In addition to the inhibitory subtypes, activating KIR isoform may maintain Norm-Preg. Interaction between the activating KIR2DL4 receptor on NK cells with HLA-G stimulates NK cells to secrete cytokines and angiogenic factors for vascular remodeling [88] and prevent the cytotoxic action of CD+8 T cells on placental tissues. HLA-G levels decrease in PE [89]. Decreased HLA-G mRNA cause insufficient growth of trophoblasts and decreased cleavage of the embryo [89], and mild PE has been associated with deficiency of HLA-G3 isoform [90].

Hence, impaired integrins and MMPs expression, aberrant cytokines and NK cells activity, and downregulation of HLA-G can reduce spiral artery remodeling and induce RUPP in PE.

Circulating Bioactive Factors Released in Response to RUPP

RUPP and placental ischemia/hypoxia could cause the release of bioactive factors into the maternal circulation leading to EC and VSM dysfunction, increased vasoconstriction and HTN. Circulating bioactive factors include pro-angiogenic/anti-angiogenic imbalance, and increased anti-angiogenic factors, pro-inflammatory cytokines, ROS, HIF-1 and AT₁-AA (Table 2, Table 3).

Pro-angiogenic/Anti-angiogenic Factors Imbalance in PE

VEGF is a major angiogenic factor that binds to tyrosine kinase receptors flt-1 (VEGFR-1) and flk-1 (KDR, VEGFR-2) on ECs to regulate EC proliferation, vasculogenesis and vascular permeability. In ECs, VEGF increases [Ca²⁺]_i, Ca²⁺/calmodulin and eNOS activity leading to decreased vessel tonicity and BP and increased angiogenesis [1]. Plasma levels of VEGF vary in PE. Free VEGF measured using radioimmunoassay or competitive enzyme immunoassay could be low in PE due to high levels of soluble fms-like tyrosine kinase-1 (sFlt-1) which captures VEGF [91]. However, bound VEGF measured using ELISAs may be higher in PE than Norm-Preg [92]. Also, during the vasoconstrictive and latent phase of PE the increased vascular shear-stress could increase circulating VEGF [3]. Mice lacking one VEGF allele in renal podocytes develop typical renal pathology of PE. Infusion of antibodies against VEGF causes 50% reduction of VEGF, glomerular endotheliosis and proteinuria similar to that seen in PE [93, 94], whereas infusion of VEGF ameliorates the renal lesions, glomerulonephritis and thrombotic microangiopathy in RUPP rats [91]. Also, *in vitro* angiogenesis studies show that VEGF or an sFlt-1 antibody can reverse the anti-angiogenic effects of sFlt-1 [95].

PlGF is a pro-angiogenic factor that has only 1/10th the affinity for Flt-1 receptor compared to VEGF, but is ~40 times higher than VEGF during Norm-Preg. PlGF dilates uterine vessels, and promotes EC growth, vasculogenesis, and placental development. PlGF stimulates Bcl-2 expression in placental extracts which contributes to angiogenesis and maintains the network of capillaries of microvascular ECs [95]. During PE, circulating PlGF levels decrease while the levels of its antagonist sFlt-1 increase [1]. PlGF has four alternatively spliced mRNA species (PlGF1-4), and its predominant isoform PlGF-1 is down-regulated in PE [92].

sFlt-1 (sVEGFR-1) is an anti-angiogenic factor expressed as an alternatively spliced variant of VEGFR-1, and lacks transmembrane and cytoplasmic domains. Trophoblast cells express sFlt-1 mRNA, and sFlt-1 level is 1.5±0.2 ng/ml in Norm-Preg compared to 0.15±0.04 ng/ml in healthy non-pregnant women. In placental hypoxia, HIF-1 may bind to the promoter region of *flt-1* gene leading to up-regulation of sFlt-1 [91, 95]. sFlt-1 levels may increase before the onset of PE or secondary to placental ischemia/hypoxia [96]. There is a decline in VEGF/sFlt-1 and PlGF/sFlt-1 ratio by 53% and 70%, respectively in PE placenta [95]. Also, the Flt-1/sFlt-1 gene is located on chromosome 13q12, and in trisomy 13 an extra copy of this gene may lead to excess circulating sFlt-1, reduced free PlGF levels, increased sFlt-1/PlGF ratio and PE [97]. The sFlt-1/PlGF ratio is higher in PE than Norm-Preg women from second trimester onwards, and can indicate the onset of PE. In twin pregnancies, circulating sFlt-1 levels and sFlt-1/PlGF ratio are twice as high as those in singleton pregnancies [98].

Also, exposure of ECs in tissue explants to PE plasma results in reduced angiogenesis, while removal of sFlt-1 restores EC function and angiogenesis [95]. Pregnant rats treated with sFlt-1 develop HTN, proteinuria, occlusion of capillaries in the renal capsule and focal fibrin deposition in glomerular cells [91].

Endoglin (Eng) or CD105, a co-receptor for TGF- β 1 and TGF- β 3, is highly expressed on cell membranes of ECs and syncytiotrophoblasts. sEng is an anti-angiogenic protein that inhibits TGF- β 1 signaling and TGF- β 1-mediated activation of eNOS and vasodilation. sEng is higher in PE than Norm-Preg women from 18 weeks onwards and continues to increase with gestational age. sEng expression also increases in placental extracts exposed to 3% O₂ compared to those exposed to 20% O₂ [99]. Mutations in *Eng* gene result in loss of capillaries, arteriovenous malformations, and hereditary hemorrhagic telangiectasia [100]. Also, sEng impairs the formation of endothelial tubes in cultured human umbilical vein endothelial cells (HUVECs) [101]. Placental endoglin increases 2 to 3 months prior to the onset of PE and is released into the maternal circulation. In RUPP rats, sEng increases with the severity of PE-like symptoms and falls after delivery [67]. Increased levels of sEng and increased sFlt-1/PIGF ratio can predict the development of PE. sEng acts in concert with sFlt-1 to induce vascular permeability, proteinuria, hemolysis elevated liver enzymes low platelets (HELLP) syndrome, IUGR and severe HTN in pregnant rats, and tissue analysis of these rats reveals glomerular endotheliosis, placental infarction, liver necrosis, and fragmented red blood cells (schistocytes) [101]. However, sFlt-1 and sEng levels may not change in PE from Norm-Preg [102].

Studies on animal models and human placenta suggest potential benefits of correcting the pro-angiogenic/anti-angiogenic factors imbalance in PE. Edaravone is a free radical scavenger that inhibits sFlt-1 expression and prevents placental vessels abnormalities in hypoxic human trophoblast cells [103]. Sildenafil citrate reduces anti-angiogenic factors in L-NAME treated rats [104]. Also, pravastatin decreases the levels of anti-angiogenic factors in pregnant mice, and other statins improve VEGF and PIGF levels in mice models [105]. Of note, statins are thought to be contraindicated in pregnancy because of the perceived increased risk of fetal congenital abnormalities. However, a recent review of studies of statins during pregnancy has suggested that the risk of major congenital abnormalities from exposure to statins in pregnancy is similar to the background risk of the population, and no specific pattern of abnormalities was observed. Also, while existing studies are small with limitations in their quality, preliminary evidence suggests a beneficial role for statins in preventing PE [106]. Although administration of VEGF could improve the proangiogenic/antiangiogenic imbalance, VEGF impairs bradykinin-mediated vascular relaxation, and may enhance basal tone and vascular permeability in PE [68], and the use of modulators of PIGF could be more promising in PE.

Circulating Cytokines in PE

Plasma levels of PAI-1, TNF- α and C-reactive protein show some increase during Norm-Preg and reach higher levels in PE [1]. Ischemic placenta may enhance the synthesis of inflammatory cytokines. An imbalance in CD4+T cells leads to increased pro-inflammatory cytokines and decreased anti-inflammatory cytokines in PE. During placental ischemia, IL-6

could disturb the balance of CD4+T cells. T helper-17 cells (ROR γ +CD4+Tcells) are a subclass of CD4+T cells that are increased in PE and secrete the inflammatory cytokine IL-17. Another subset of CD4+T cells called Treg cells which express transcription factor forkhead box P3 (FoxP3) and hence called FoxP3+Tcells have anti-inflammatory properties and are decreased in PE. IL-17 stimulates the production of other cytokines such as IL-6, IL-8, TNF- α , granulocyte colony stimulating factor, and macrophage colony stimulating factor in PE [107]. Norm-Preg rats treated with IL-17 exhibit decreased pup and placental weight and increased levels of Th-17 cells, placental oxidative stress, AT₁-AA, BP and ROS which are attenuated with the SOD analogue Tempol, suggesting ROS as a signaling molecule of IL-17 [82, 108].

There is a 2-fold increase in TNF- α in the plasma of PE compared to Norm-Preg women, and in the renal cortex and medulla of the RUPP rats. TNF- α may work in concert with other inflammatory cytokines to increase ET-1 levels and cause HTN in RUPP rats [83]. Infusion of LIGHT, a member of TNF family, increases BP and ET-1 expression in both non-pregnant and pregnant rats and induces sFlt-1 expression and causes proteinuria in pregnant rats [85].

IL-6 is a pro-inflammatory cytokine which increases AT₁-AA, and infusion of IL-6 in pregnant rats decreases renal plasma flow, and causes proteinuria and HTN [109]. Chronic infusion of TNF- α or IL-6 in late pregnant rats leads to increased BP, enhanced vascular contraction and reduced endothelium-dependent relaxation via the NO-cGMP pathway [110, 111]. Also, IL-6 binds to the surface receptor GP-130 on ECs and stimulates dimerization of the receptor leading to abnormal intracellular signaling and vascular dysfunction. IL-6 also disrupts the tight junctions in ECs and increases vascular permeability [84].

IL-1 β is a pro-inflammatory cytokine that contributes to the inflammatory response and disrupts ECs in PE. Serum IL-1 β and its natural inhibitor, IL-1 receptor antagonist (IL-1Ra) are increased in PE, and women homozygous for allele 2 of the IL-1Ra gene (IL-1RN*2) have more severe form of PE than women with other IL-1RN genotypes [112].

IL-10 is an anti-inflammatory cytokine that induces corpus luteum maturation and progesterone production to maintain Norm-Preg [1]. Administration of IL-10 antibodies increases BP in pregnant baboons [113].

Cytokines could be potential targets in the management of PE. Etanercept is a TNF- α inhibitor that lowers BP, increases eNOS expression, decreases ET-1 levels and prevents cardiac changes in RUPP rats [83, 114]. IL-17 soluble receptor C could inhibit IL-17 and prevent the recruitment of host defense cells and in turn suppresses the development of inflammatory response, decreases AT₁-AA and ROS, and ameliorates HTN and improves pup and placental weight in RUPP rats [82].

Reactive Oxygen Species (ROS) in PE

Reactive Oxygen Species (ROS) such as superoxide (O₂^{•-}), hydrogen peroxide (H₂O₂), hydroxyl ion (OH[•]) contain highly reactive O₂. Norm-Preg is a state of oxidative stress in which placental production of ROS is counterbalanced by abundant antioxidants. During PE,

decreased expression of antioxidants such as heme oxygenase-1 (HO-1), HO-2, copper/zinc superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase fails to counterbalance the increased ROS production leading to lipid peroxidation, increased TXA₂ and loss of GPx activity in placenta [1]. In PE, reduced brachial artery flow-mediated endothelium-dependent vasodilation is associated with decreased plasma levels of the antioxidant ascorbate and is ameliorated with administration of ascorbic acid [115]. Neutrophils isolated from women with PE release ROS specifically O₂^{•-} which could dominate NO production from neutrophils and ECs. Also, advanced glycation end products (AGE) produce ROS and mediate vascular dysfunction in PE [62].

The reduced blood flow in the spiral arteries associated with RUPP also mediates an ischemia/hypoxia-reperfusion injury leading to oxidative changes in placental proteins and lipids, mitochondrial injury, tissue apoptosis and increased ROS production [116]. ROS decreases the amount of stored Ca²⁺ and intracellular ATP and in turn leads to endoplasmic reticulum (ER) stress and production of misfolded proteins that the cell attempts to fix via the unfolded protein response (UPR). During low-grade ER stress the UPR phosphorylates eukaryotic initiation factor 2, prevents the binding of the initiator to the ribosomes and thereby reduces cell proliferation and maintains cell survival. However, severe ER stress prolongs the UPR exposure during pregnancy which makes the syncytium undergo apoptosis [117] and causes placental remnants to enter the maternal blood and stimulate the production of cytokines and free radicals leading to EC dysfunction and PE [116]. In PE, ROS attack polyunsaturated fatty acids in the cell membrane converting them into lipid peroxides which enter the maternal circulation and cause abnormal expression of endothelial adhesion molecules, change the membrane fluidity and permeability and cause EC damage [73, 118]. Also, in PE, O₂^{•-} and peroxynitrite (ONOO⁻) stimulate MMP-1, -2, and -9 and degrade HIF-1 α in VSM leading to vascular dysfunction. ROS also stimulate Ca²⁺ release from the intracellular stores, influx of extracellular Ca²⁺, PKC and Rho-A/Rho-kinase signaling and VSM contraction.

Hypoxia-Inducible Factor (HIF) in PE

HIF-1 is a transcriptional activator and a heterodimer consisting of O₂-regulated HIF1- α and HIF2- α subunits and a constitutively expressed HIF1- β subunit. More than 100 genes downstream from HIF-1 including VEGF, leptin, TGF- β 3, and NOS perform different cell functions, and DNA microarrays show that HIF-1 regulates more than 2% of all human genes in arterial ECs, directly or indirectly. In hypoxia, HIF-1 binds to 50-base pair cis-acting hypoxia-responsive element of various genes at their enhancer and promoter regions leading to the transcription of these genes. HIF-1 α and HIF-2 α proteins are increased in PE placenta. HIF-1 α accumulates in the placenta due to increased formation secondary to ischemia/hypoxia or decreased degradation after reperfusion/oxygenation as a result of proteosomal dysfunction [119]. HIF-1 α upregulates sFlt-1 and sEng, binds to ET-1 gene, contributes to shallow trophoblast invasion and induces ACE expression in the lungs and kidney during PE [120].

AngII and AT₁ Receptor Autoantibodies (AT₁-AA)

AngII is an important regulator of electrolyte homeostasis and BP. About 40% of AngII is produced locally in the placenta by chymase, a chymotrypsin-like serine protease, which is a non-angiotensin converting enzyme found mainly in the syncytiotrophoblast of placenta. AngII via AngII type 1 receptor (AT₁R) promotes vasoconstriction, vascular growth, and inflammation, and increases $[Ca^{2+}]_i$ and Rho/Rho-kinase activity in VSM. AngII via endothelial AT₂R activates eNOS, and increases production of NO and PGI₂ which oppose AngII-induced vasoconstriction. Although Norm-Preg is associated with increased plasma levels of renin and AngII, the response to AngII is decreased due to decreased expression of AT₁R possibly by AT₂R. In PE, AT₁R forms a heterodimer with bradykinin B2 receptor and becomes hyper-responsive to AngII. Hypoxia increases AT₁R expression and plasma levels of AngII in rabbits [121] and increases AngII, renin, and ACE mRNA in PE placenta [122]. Increased plasma hemopexin activity downregulates AT₁R in human monocytes and ECs in pregnant rats, and inhibition of hemopexin during PE enhances AT₁R expression and vasoconstriction [123].

AT₁-AA is a bioactive factor that mediates vascular signaling via protein-1, calcineurin, and nuclear factor kappa B (NF κ B). AT₁-AA induces the secretion of PAI-1 which reduces trophoblast invasion, increases ROS, increases $[Ca^{2+}]_i$, activates the tissue factor causing thrombosis, increases BP, causes vascular damage to the adrenal glands, and reduces aldosterone secretion in sFlt-1 treated pregnant mice [124, 125]. Infusion of CD4+ T cells obtained from women with PE in pregnant rats stimulates the immunoglobulin from B-cells which in turn increases AT₁-AA production, and inhibition of B-cells attenuates AT₁-AA mediated HTN in these rats [126]. AT₁-AA could be an important target in PE. AT₁-AA binds to a 7 amino acid sequence in the second extracellular loop of the AT₁R, and a 7 amino acid antibody blocking epitope peptide could prevent AT₁-AA from binding to AT₁R [125]. The sequence, AFHYESQ, in the 7 amino acid sequence of AT₁R has been shown to block AT₁-AA-induced stimulation of cardiomyocyte contraction in rats [127]. Also, VEGF₁₂₁ corrects AT₁-AA-mediated increase in sFlt-1, and prevents impairment in the vasculature of the adrenal glands and increases production of aldosterone in AT₁-AA treated pregnant mice [124].

Catechol-O-methyl transferase (COMT)

COMT is an enzyme that converts 17-hydroxyestradiol into 2-methoxyestradiol (2-ME). 2-ME is elevated during the third trimester of Norm-Preg but is reduced in placenta of women with PE. Also, low levels of COMT and 2-ME are associated with PE-like condition in pregnant mice likely due to upregulation of HIF- α , as administration of 2-ME lowers HIF- α and ameliorates PE-like features in COMT-deficient mice [120]. 2-ME also suppresses placental hypoxia and sFlt-1 levels in PE. 2-ME levels in plasma and urine may be used as a diagnostic marker for PE, and 2-ME may serve as a therapeutic option in PE [128].

Vascular Targets in PE

Circulating bioactive factors target several tissues and organs including the vasculature, kidney and brain. In the vasculature, bioactive factors could decrease endothelium-derived relaxing factors such as NO and increase contracting factors such as ET-1 or cause

imbalance in the prostaglandins. Bioactive factors could also cause changes in VSM contraction mechanisms including Ca^{2+} , PKC, mitogen-activated protein Kinase (MAPK) and Rho-Kinase. The changes in these vascular mediators would lead to decreased vasodilation, and increased vasoconstriction and vascular resistance during PE (**Figure**).

Alterations in NO in PE

Nitric Oxide (NO) is a potent vasodilator and VSM relaxant produced in response to increased blood flow. The NO synthase (NOS) isoforms neuronal nNOS or type 1, inducible iNOS or type 2 and endothelial eNOS or type 3 convert L-arginine to L-citrulline and form NO. During Norm-Preg, increased NOS expression/activity in human uterine artery increases NO levels [129]. NO activates cGMP, a second messenger of NO and cellular mediator of VSM relaxation. cGMP levels are increased in plasma and urine of Norm-Preg women particularly during the first trimester when the maternal circulation undergoes rapid dilation [130].

The expression of eNOS mRNA and protein and NO production decrease during hypoxia *in vivo* [131]. Measurements of total NO production during HTN-Preg and PE have shown variable results ranging from decreased levels [132] to increased levels of nitrate/nitrite [133], and the disparity could be related to dietary nitrate intake. However, a study conducted on women with PE with carefully controlled dietary nitrate/nitrite intake did not show decreased NO production [134]. Also, NOS expression may be similar in Norm-Preg and PE placentas [135]. While the whole-body NO may not change in PE, the decrease in vascular relaxation suggests tissue-specific differences in NOS expression and NO bioavailability. For instance, in late pregnant rats, renal eNOS decreases by 39% while iNOS and nNOS increase by 31% and 25%, respectively [136]. Also, ascorbate is essential for decomposition of S-nitrosothiols and the release of NO, and ascorbate deficiency may lead to increased plasma levels of total S-nitrosothiols and S-nitrosoalbumin and decreased NO release in PE. [137].

During placental ischemia, oxidative stress reduces L-arginine in the platelets [138] while up-regulation of arginase enzyme may cause L-arginine to form urea instead of NO, thus decreasing NO in PE [139]. Also, hypoxia may induce an uncoupling reaction due to reduction in the NOS co-factor tetrahydrobiopterin (BH4) leading to ROS production and decreased NO formation [138]. $\text{O}_2^{\bullet-}$ reacts with NO to form peroxynitrite which not only decreases NO activity but also causes reduced trophoblast growth and endothelial dysfunction in PE.

Experimental studies have shown that NO increases VEGF and PlGF and decreases sFlt-1 in hypoxic human trophoblast cells [140]. NOS blockade with N^ω-nitro-L-arginine methyl ester (L-NAME) during mid- to late-gestation in pregnant rats produces PE-like syndrome manifested as increased ET-1 activity, high BP, renal vasoconstriction, proteinuria, thrombocytopenia and IUGR [141]. Also, RUPP in pregnant rats reduces renal expression of nNOS leading to changes in renal hemodynamics and HTN [66].

Increased Endothelin-1 in PE

ET-1 is a major vasoconstrictor that may play a role in PE. Several factors including cytokines, hypoxia and AT₁-AA stimulate ECs to produce ET-1 [142]. ET-1 levels reach 2- to 3-fold higher in the plasma [143], 4- to 8-fold higher in umbilical cord cells [144] and increase in renal tissues during later stages of PE, and return to normal levels within 48 hrs of delivery [145]. ET-1 may be involved in the progression rather than the initiation of PE [146]. Preproendothelin levels show 45% increase in renal cortex and 22% increase in the medulla of RUPP rats [147]. Also, infusion of AT₁-AA in pregnant rats causes increases in BP that are attenuated with the AT₁R antagonist losartan or an endothelin receptor antagonist [148].

ET-1 activates endothelin type A receptor (ET_AR) in VSM which may be increased in RUPP rats, and ET_BR in ECs and renal cells which may be reduced in RUPP rats [149]. ET-1 induces apoptosis of trophoblast cells, reduces spiral artery remodeling, increases the oxidant malondialdehyde, decreases anti-oxidants such as glutathione, glutathione disulfide and ascorbic acid [118], increases sFlt-1, and reduces VEGF in PE [142]. Increased expression of ET-1, ET_AR or ET_B2R in VSM of renal arterioles may counteract the vasodilator effects of the pregnancy hormone relaxin [150]. ET-1 via ET_AR stimulates Ca²⁺ release from the intracellular stores and Ca²⁺ entry through Ca²⁺ channels and causes PKC-dependent inhibition of K⁺ channels leading to increased [Ca²⁺]_i and VSM contraction. In hypoxia, up to 80% of ET-1 induced vascular contraction occur independently of [Ca²⁺]_i and PKC [151]. ET-1 also activates ET_B1R in ECs and stimulates the release of NO, PGI₂, and EDHF [152] that reduce myogenic vascular tone, promote vasodilation of renal arteries and hyperfiltration in pregnant rats [134], and downregulation of ET_BR may impair trophoblast invasion in PE, and decrease microvascular vasodilator activity in pregnant rats [149].

Prostaglandins Imbalance PE

A decrease in production of prostacyclin (PGI₂) and an increase in production of thromboxane A₂ (TXA₂) may cause vascular dysfunction in PE [153]. PGI₂ is an anti-platelet aggregator and a vasodilator produced from the metabolism of arachidonic acid by cyclooxygenase-1 (COX-1) and COX-2. Endothelium-derived PGI₂ contributes to the hemodynamic and vascular changes during pregnancy, and the plasma and urinary levels of 6-keto-prostaglandin F₁α (PGF₁α), a hydration product of PGI₂, increase in Norm-Preg. Hypoxia may cause downregulation of COX-1 with consequent reduction in endothelium-derived PGI₂. Women with PE have reduced levels of 6-keto PGF₁α and 25% reduction in 6-keto PGF₁α/TXA₂ ratio as compared to Norm-Preg women [154]. Other studies show similar release of PGI₂ in both apical and basal trophoblast cells of PE and Norm-Preg women while the release of TXA₂ from basal trophoblast cells is increased in PE [155].

TXA₂ is a vasoconstrictor that stimulates platelet aggregation, VSM cell proliferation and mitogenesis. TXA₂ prostanoid (TP) receptors activate Ca²⁺, PKC, MAPK and Rho-kinase in VSM to cause vasoconstriction [156]. Increased production of lipid peroxides may activate COX-1 which increases TXA₂ synthesis in PE [153]. TxB₂ metabolites, markers of TXA₂ synthesis, are higher in PE than Norm-Preg women [157] and platelet TXA₂ synthesis is

increased in severe PE. Some studies suggest that TXA₂ may not contribute to HTN and renal vasoconstriction in RUPP rats [158], and show decreased effects of TXA₂ analogs on the uterine artery of pregnant guinea pigs [159], while other studies show that infusion of TXA₂ analogs in pregnant rats [160] and rabbits [161] leads to HTN. It has been suggested that low dose aspirin (60 to 150 mg) may cause 24% reduction in the risk of developing PE, reduce TXA₂ mediated damage in PE, and may not cause placental abruption, postpartum hemorrhage or fetal intracranial bleeding in pregnant women [162]. Also, ozagrel, a TXA₂ modulator may reduce HTN-Preg and proteinuria in PE [163]. However, no beneficial effects of low dose aspirin were seen in women at higher risk of PE [164], and aspirin may cause adverse effects in pregnant women such as post-partum bleeding and epigastric pain [165].

Endothelium-Derived Hyperpolarizing Factor (EDHF)

EDHF is an important relaxing factor particularly in small resistance vessels. While NO and PGI₂ are the dominant vasodilators in male mice, EDHF may be the predominant vasodilator in female mice [166]. Increased [Ca²⁺]_i in ECs may activate small conductance (SK_{Ca}) and intermediate conductance Ca²⁺-activated K⁺ channels (IK_{Ca}) and lead to EDHF-mediated dilation in uterine radial arteries of pregnant rats [167]. Also, PGI₂ binds to VEGFR-1 and activates endothelial SK_{Ca} in mesenteric arteries of pregnant rats to cause EDHF-mediated activation of large conductance Ca²⁺-activated K⁺ channels (BK_{Ca}) in VSM [168, 169]. The delayed rectifier type of voltage-sensitive K⁺ channels (K_v) may also play a role in EDHF-mediated dilation of uterine artery of pregnant rats [170]. Estrogen can modulate EDHF activity and vascular tone, and EDHF contributes to ACh-induced dilation of radial uterine arteries of non-pregnant rats treated with estrogen [171]. EDHF causes greater dilation in the smaller distal than the bigger proximal uterine vessels [167]. In subcutaneous arteries from women with PE myoendothelial gap junctions alone or in combination with H₂O₂ or CYP450 epoxygenase metabolites of arachidonic acid mediate EDHF-induced vasodilation, and EDHF activity via myoendothelial gap junctions may be disrupted in PE [172].

Heme Oxygenase (HO)

HO may be involved in the proper placentation and maintenance of pregnancy. Two isoforms of HO have been identified in humans and rodents, inducible HO-1 and constitutive HO-2. Several factors including heavy metals, oxidative stress, UV irradiation, and lipopolysaccharide induce HO-1 expression. HO-1 is upregulated in the uterus and placenta during pregnancy [173]. One of the products of HO is bilirubin/biliverdin which has antioxidant effects. Another product of HO is carbon monoxide (CO), which at low concentration dilates the vessels, and promotes angiogenesis. HO decreases sFlt-1 and hypoxia-induced oxidative stress, and attenuates HTN in PE [174]. There is 30% decrease of HO in hypoxic as compared to normal placental villi [175]. In pregnant mice, HO-1 deficiency results in decreased litter size, thinner placenta and apoptosis, and higher BP and sFlt-1 levels, and HO-2 levels may be elevated as a compensatory response [173]. HO-induced production of CO may be useful in management of PE. CO reduces apoptosis of trophoblast cells, dilates placental vessels, increases vascularity and reduces the apo-necrotic pathway in the placental villous explants, thereby limiting the release of placental debris in

the maternal circulation during PE [116]. CO alleviates HTN and proteinuria in adenovirus sFlt-1 PE-like mouse model [176], and the HO inducer cobalt protoporphyrin (CoPP) reduces BP in HTN-Preg RUPP rats [177].

VSM Ca^{2+} in PE

Ca^{2+} is a major determinant of VSM contraction and growth. Ca^{2+} release from the intracellular stores and Ca^{2+} entry from the extracellular space increase $[\text{Ca}^{2+}]_i$ in VSM. Ca^{2+} binds calmodulin (CAM) to form Ca^{2+} -CAM complex which activates myosin light chain (MLC) kinase, causes MLC phosphorylation, initiates actin-myosin interaction and produces VSM contraction. Decreased Ca^{2+} in VSM activates MLC phosphatase to dephosphorylate MLC and dissociates the Ca^{2+} -CAM complex. Endothelium-derived relaxing factors act on VSM to decrease $[\text{Ca}^{2+}]_i$. In hypoxia, decreased relaxing factors reduce VSM Ca^{2+} extrusion and increased contracting factors increase $[\text{Ca}^{2+}]_i$ and cause VSM contraction. In Norm-Preg, increased K_{Ca} channel activity decreases uterine artery tonicity and increases uteroplacental blood supply. In PE, K_{Ca} channel activity is suppressed leading to increased uterine artery $[\text{Ca}^{2+}]_i$, vasoconstriction and reduced fetal blood supply [178]. Myometrial vessels may show similar vasoconstriction response to high KCl, phenylephrine and AngII in Norm-Preg and PE [179]. However, basal and agonist-stimulated $[\text{Ca}^{2+}]_i$ are reduced in renal arterial VSM of Norm-Preg rats, and increased in pregnant rats treated with L-NAME [180]. AT_1R increases $[\text{Ca}^{2+}]_i$ in the platelets, erythrocytes, and lymphocytes of PE women, and these effects subside after 6 weeks of delivery [125]. AngII- and caffeine-induced contraction and $[\text{Ca}^{2+}]_i$ in Ca^{2+} -free solution are similar in VSM of Norm-Preg and RUPP rats while AngII- and KCl-induced maintained $[\text{Ca}^{2+}]_i$ in a Ca^{2+} -containing medium is greater in VSM of RUPP than Norm-Preg rats, suggesting that it is not the release of intracellular Ca^{2+} but the increase in Ca^{2+} entry from extracellular space that increases vasoconstriction in HTN-Preg [181].

Protein Kinase C (PKC) in PE

PKC is an important mediator of VSM contraction. PKC phosphorylates CPI-17 which inhibits MLC phosphatase and in turn increases MLC phosphorylation and VSM contraction. PKC also phosphorylates calponin, an actin binding protein which inhibits myosin ATPase leading to more actin-myosin interaction and VSM contraction. Phorbol esters activate PKC to cause VSM contraction with no detectable change in $[\text{Ca}^{2+}]_i$, suggesting that PKC increases Ca^{2+} sensitivity of the contractile proteins. PKC activity and contraction are reduced in uterine artery of late pregnant ewes and gilts and aorta of late pregnant rats [182-184]. Also, the expression and subcellular redistribution of Ca^{2+} -dependent α -PKC and Ca^{2+} -independent δ - and ζ -PKC are reduced in aortic VSM of late pregnant rats, but increase in L-NAME treated rats [183, 185]. PKC may increase the production of AT_1 -AA which stimulates AT_1R . In cultured rat cardiomyocytes treatment with IgG obtained from PE women enhances AT_1R -mediated response which is ameliorated with the PKC inhibitor calphostin C [127]. Increased BK_{Ca} channel activity may inhibit PKC-mediated contraction in ovine uterine arteries during pregnancy, and gestational hypoxia may upregulate PKC and inhibit BK_{Ca} in ovine uterine arteries [178]. PKC inhibitors decrease TXA_2 mediated contraction in uterine and mesenteric arteries of non-pregnant rats and in mesenteric of pregnant rats, supporting a role of PKC in mediating

VSM contraction during pregnancy [156]. Blocking PKC can prevent PKC mediated vasoconstriction in PE. Cicletanine is an anti-hypertensive compound that may prevent the increase in PKC and lower BP in HTN-Preg rats.

MAPK is a serine/threonine protein kinase that regulates cellular activities such as gene expression, mitosis, differentiation, and VSM contraction. During VSM contraction, PKC may phosphorylate MAPK kinase which in turn phosphorylates and activates MAPK. Activated MAPK phosphorylates the actin-binding protein caldesmon thus preventing its inhibition of ATPase and increases actin-myosin interaction and VSM contraction. Changes in PKC activity in VSM during Norm-Preg and HTN-Preg could affect MAPK/caldesmon phosphorylation and VSM contraction [186]. This is supported by reports that inhibition of p38 MAPK reduces TXA₂-induced contraction in uterine and mesenteric arteries of virgin and pregnant rats [156].

Rho-Kinase in PE

Rho is a family of small GTP-binding proteins that are involved in cell migration, cytoskeletal reorganization and VSM contraction. Rho-kinase is activated by GTP binding and inactivated by hydrolyzing GTP to GDP. There are two isoforms of Rho-kinase, rock I (ROK β) and rock II (ROK α), which may be important in the formation of microvilli structures during pregnancy. Human placental villi show abnormal expression of rock II and apoptosis of the syncytium in PE [187]. Rho-kinase increases Ca²⁺ sensitivity of the contractile proteins in subcutaneous resistance arteries of PE women [188]. Also, AngII via AT₁R may induce RhoA/Rho-kinase activity in L-NAME treated HTN rats [189]. Rho-kinase may stimulate IL-17 to phosphorylate the inhibitory eNOS Thr495 residue thus decreasing NO production in PE [190]. Inhibition of Rho-kinase reduces TXA₂-induced contraction in uterine vessels of non-pregnant rats [156]. However, some studies show decreased expression of Rho-kinase in umbilical arteries of PE women [191].

Matrix Metalloproteinases (MMPs) and ECM in PE

MMPs are important regulator of ECM remodeling during pregnancy. In PE, abnormal MMPs expression/activity may cause vascular dysfunction. The PE-induced changes in MMPs could be due to increased levels of peroxynitrite, since reduction of peroxynitrite in RUPP rats decreases MMP-mediated endothelium dysfunction. MMPs increase cytokines and ROS, induce proteolysis of VEGF receptors, increase vasoconstrictors such as ET-1 and decrease vasodilators in PE [76], and increase ET-1 levels in RUPP rats [192]. MMP-2 is the main collagenolytic enzyme in umbilical cord artery (UCA) wall that cleaves big ET-1 into ET-11–32 which is a potent vasodilator, and inhibits relaxin and thrombin in UCA. Some studies suggest an increase in MMP-2 levels [193] while other studies suggest decreased levels of MMP-2 in UCA [194]. Hypoxia increases MMP-9 mRNA and enzymatic activity 3- to 4-fold in HTN-Preg compared with Norm-Preg [195]. MMPs may have a bidirectional relationship with VEGF such that VEGF upregulates MMP expression while membrane MMP-1 promotes VEGF expression [196]. Pappalysin-2 (PAPP-A2), a protease that cleaves IGF binding proteins, is higher in PE than in Norm-Preg [197]. MMPs could be an important target in the management of PE. Ca²⁺ channel blockers such as lercanidipine and amlodipine decrease plasma MMP-9 levels/activity, while felodipine increases MMP-2 levels in HTN

patients. Similar effects of Ca^{2+} channels blockers on MMPs were also observed in HTN rodent models. Doxycycline, a non-specific MMP inhibitor, is thought to alleviate HTN and vascular dysfunction in PE, but has been shown to decrease placenta weight and cause IUGR in both Norm-Preg and HTN-Preg rats, and reduce trophoblast invasion and placental perfusion in HTN-Preg rats [76].

Management of PE

There are few options available for management of PE including induction of labor, anti-hypertensive drugs and Mg^{2+} sulfate. Currently, induction of labor is the most effective management of PE. Some indications of delivery in women with PE include uncontrolled HTN, gestational age exceeding term, HELLP syndrome, deteriorating renal function, placental abruption, eclampsia, acute pulmonary edema, and severe IUGR. Induction of labor through the vaginal route within 24 hours results in better maternal and fetal outcomes in PE. Misoprostol tablets, a synthetic PGE1 analog, and trans-cervical Foley catheterization induce vaginal delivery in 60% and 41% of women with PE, respectively [198]. Graduated compression stockings decrease the risk of deep venous thrombosis and low molecular weight heparin ameliorates nephrotic syndrome [1].

Anti-hypertensive drugs are used to correct the increased BP in PE [199, 200]. Methyldopa is considered as the first-line oral anti-hypertensive medication in PE. Methyldopa is a centrally acting α_2 -adrenergic agonist prodrug, which is metabolized to α -methyl norepinephrine and then replaces norepinephrine in the neurosecretory vesicles of adrenergic nerve terminals. Therefore, methyldopa could function as a sympatholytic agent to inhibit sympathetic impulses from the medulla oblongata in the brain stem to the blood vessels, thus reducing vasoconstriction and BP. Second line anti-hypertensive drugs are used if methyldopa fails or if the patient develops tolerance to methyldopa, and include labetalol, nifedipine and hydralazine. Labetalol is a mixed α/β -adrenergic receptor antagonist that blocks vascular adrenergic receptors and reduces vasoconstriction. Nifedipine is a Ca^{2+} channel blocker that reduces VSM $[\text{Ca}^{2+}]_i$ and Ca^{2+} -dependent contraction. Hydralazine is usually used intravenously in severe case of elevated BP. Some antihypertensive drugs are contraindicated during pregnancy, especially in the second and third trimesters. Angiotensin receptor blockers (ARBs) such as losartan and angiotensin-converting enzyme (ACE) inhibitors such as enalapril and captopril could be associated with fetal IUGR and birth defects such as fetal hypocalvaria, cardiac and renal defects, neonatal anuric renal failure, and fetal and neonatal death. Diuretics such as furosemide and hydrochlorothiazide could decrease blood volume and cause electrolyte imbalance due to excessive salt and water excretion. Also, while propranolol could be effective in lowering BP and reducing heart rate and cardiac output due to its β -adrenergic blocking effects, it may decrease uteroplacental blood flow, impair fetal response to hypoxic stress, and may be associated with fetal bradycardia, IUGR, and neonatal hypoglycemia [199, 200].

Among women with PE, signs of eclampsia include severe headache (especially occipital headache), brisk reflexes (3+), papilledema, and visual disturbances which require immediate maintenance of airway patency, prevention of fluid aspiration and anticonvulsants [1]. Mg^{2+} sulfate is often used to prevent and treat eclamptic seizures and

causes 60% reduction in the risk of developing eclampsia and 50% decrease in the development of recurrent episodes of eclampsia in women with PE [201]. Mg^{2+} sulfate dilates blood vessels, maintains the integrity of the blood-brain barrier, protects against cerebral ischemia, and decreases the levels of AngII, ET-1 [109] and the pro-inflammatory cytokines TNF- α , IL-6 and IL-1 β in PE [201].

Conclusions and Future Directions

Although the causes of HTN-Preg and PE have not been clearly defined, recent observational, clinical and experimental studies have shed some light on the mechanisms of this enigmatic pregnancy-associated disorder. Demographic, genetic, and environmental factors may induce localized abnormalities in integrins, MMPs, and inflammatory cells in the placenta leading to placental ischemia/hypoxia and the release of biologically active factors such as cytokines. RUPP-induced increase in TNF- α further aggravates placental ischemia, promotes the release of antiangiogenic factors such as sFlt-1, and stimulates the maternal defense cells to release other pro-inflammatory cytokines, immunoglobulins, ROS, and AT₁-AA [85, 202]. These bioactive factors target blood vessels, decrease vasodilators such NO, and increase vasoconstrictors such as ET-1, leading to severe vasoconstriction, increased systemic vascular resistance and HTN-Preg. Further understanding of the bioactive factors, vascular mediators and cellular mechanisms should help design more specific and efficient measures for early detection, prevention and management of PE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations

AngII	angiotensin II
AT₁R	angiotensin II type 1 receptor
AT₁-AA	AngII AT ₁ R autoantibodies
BMI	body mass index
BP	blood pressure
[Ca²⁺]_i	intracellular free Ca ²⁺ concentration
cGMP	cyclic guanosine monophosphate
COX	cyclooxygenase
EC	endothelial cell
ECM	extracellular matrix

EDHF	endothelium-derived hyperpolarizing factor
eNOS	endothelial nitric oxide synthase
ET-1	endothelin-1
HIF	hypoxia-inducible factor
HELLP	hemolysis elevated liver enzymes low platelets
HO	heme oxygenase
HTN-Preg	hypertension in pregnancy
IL	interleukin
IUGR	intrauterine growth restriction
L-NAME	N ^ω -nitro-L-arginine methyl ester
2-ME	2-methoxy estradiol
MMP	matrix metalloproteinase
NO	nitric oxide
Norm-Preg	normal pregnancy
O₂^{•-}	superoxide anion
PE	preeclampsia
PGI₂	prostacyclin
PKC	protein kinase C
PIGF	placental growth factor
ROS	reactive oxygen species
RUPP	reduced uterine perfusion pressure
sEng	soluble endoglin
sFlt-1	soluble fms-like tyrosine kinase-1
TNF-α	tumor necrosis factor-α
TXA₂	thromboxane A ₂
VEGF	vascular endothelial growth factor
VSM	vascular smooth muscle

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Figure.

Pathophysiological mechanisms of hypertension in pregnancy (HTN-Preg) and preeclampsia. Maternal demographic, genetic, and environmental factors lead to localized abnormalities in placental cytoactive factors such as integrins, MMPs, cytokines and MHC molecules leading to shallow placentation and RUPP. RUPP-induced placental ischemia/hypoxia triggers the release of biologically active factors such as antiangiogenic factors causing antiangiogenic/proangiogenic factors imbalance, cytokines, ROS, HIF-1 and AT₁-AA or cause a decrease in 2-methoxyestradiol (2-ME). Bioactive factors in turn cause generalized decrease in vasodilators such as NO, PGI₂ and EDHF, increased release of vasoconstrictors such as ET-1 and TXA₂, increased VSM Ca²⁺, PKC, MAPK and Rho-kinase, and abnormalities in MMPs-mediated ECM and vascular remodeling, leading to increased vasoconstriction, vascular resistance and HTN-Preg.

Table 1**Representative Genes Associated with PE**

Genes	Variants	Potential Role in Preeclampsia	Ref
Maternal Genes			
Angiotensinogen (AGT)	rs699 and rs4762 and M235T polymorphism	Converted to AngI by renin	[203]
Angiotensin converting enzyme (ACE)	D (deletion) allele of (ACE) rs4646994 variant	Increases the activity of ACE which converts AngI to AngII leading to HTN.	[204]
Coagulation factor II (F2)	A allele of F2 rs1799963	Causes higher prothrombin levels. Increases thrombosis.	[204]
Coagulation factor V (F5)	rs6025, rs6020; R485K and M385T polymorphism	Prevents activation of protein C, and promotes hemostasis and thrombosis.	[204, 205]
Cytotoxic T-lymphocyte-associated protein 4	G allele of (CTLA4) rs231775 variant	Reduce surface expression of CTLA-4 and increase T-cell proliferation.	[204]
HLA-G	1597 C allele of HLA-G	Down-regulates HLA-G1 and HLA-G5 preventing the development of maternal-fetal tolerance.	[206]
Lipoprotein lipase (LPL)	rs1800590 and G allele of rs268	Causes dyslipidemia and endothelial dysfunction.	[204]
Methylenetetrahydrofolate reductase (MTHFR)	rs1801133 and T677C polymorphism	Produces enzyme that converts homocysteine to methionine	[204, 207]
NOS3	27 bp-VNTR in intron 4, rs2070744, rs1799983; C786T and T894G	Synthesizes NO in endothelium and maintains vascular homeostasis, may decrease in PE.	[204, 208]
Pappalysin-2, Pregnancy-associated plasma protein A2 (PAPP-A2)	-	Encodes proteases that cleave insulin-like growth factor (IGF)-binding proteins, resulting in local activation of IGF signaling pathways.	[197, 209]
Plasminogen activator inhibitor-1 (PAI-1)	4G allele variant rs1799889	Elevates plasma levels of PAI-1, which inactivates plasmin, hence reducing trophoblast invasion.	[204]
VEGF	T allele VEGF 936C/T	Decrease VEGF and contributes to vascular endotheliosis.	[14]
Placental Genes			
APOC1	-	Causes endothelial dysfunction and lipid peroxidation.	[16]
BAGS and Heat Shock Protein HSPA1	-	Cause Hsp70 to form antigenic peptides that stimulate dendritic cell maturation and cytokines release.	[16]
Fas	670 G variant of Fas	Reduces Fas production on T lymphocytes thus reducing T cell apoptosis and increase trophoblasts apoptosis.	[17]
HIF-1 α	-	Stimulates sFlt-1 and sEng which disrupt endothelium.	[119]
Leptin	AA genotype of the rs2167270 locus (G19A) at promoter region	Activates sympathoadrenal system which leads to HTN and inadequate placentation.	[16]
Long non-coding RNA 284	-	Binds transcription factors and inhibits gene expression.	[16]
Sialic acid binding Ig-like lectin 6 (Siglec-6)	-	Receptor of Leptin. Rate of expression is related to progression of labor.	[197, 210]
Fetal Genes			
Cyclin-dependent kinase inhibitor (cdkn1c)	-	Induces changes in placenta characteristic of RUPP.	[12]
Endoplasmic reticulum aminopeptidase 2	rs2549782 and rs17408150	-	[21]
HLA-C	Homozygous genotype HLA-C2	Acts on KIR2DL1 receptors on uterine NK cells, inhibits NK cells, and prevents trophoblast invasion.	[18]

Genes	Variants	Potential Role in Preeclampsia	Ref
HLA-G	+14/+14 HLA-G genotype	Decrease HLA-G and maternal-fetal tolerance.	[19, 89]

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Table 2

Plasma Levels of Bioactive Factors and Vascular Mediators during Normal Pregnancy (Norm-Preg) and Preeclampsia in Humans

Factor	Normal Pregnancy	Preeclampsia	Ref
Serum VEGF (pg/ml) Free	120 184 ~14	160 396 Mild ~5, Severe ~2.5	[211] [212] [91]
Free PIGF (pg/ml)	~500 780	Mild:~200, Severe:~90 202	[91] [213]
Serum sFlt-1 (ng/ml) pg/ml	~7.5 1616	~22 2521	[124] [213]
sEng (ng/mL)	9.8	46.4	[96]
Serum TNF- α (pg/ml)	0.93 $\pm$ 0.07 3.31	1.39 $\pm$ 0.09 4.68	[202] [214]
Placental IL-1 β pg/(mL.g ⁻¹)	3.59 $\pm$ 1.17	11.48 $\pm$ 2.74	[112]
IL-6 pg/mL	4.9 $\pm$ 1.1	16.5 $\pm$ 2.1	[215]
Reactive Oxygen Species (ROS) O ₂ ⁻ (nM) H ₂ O ₂ (nM)	1.6 1.4	2.2 1.8	[55]
Plasma Superoxide Dismutase (SOD) Erythrocyte SOD (U/g Hb) Plasma Catalase	6.7 $\pm$ 3.1 4834.7 1.0 $\pm$ 0.5	3.4 $\pm$ 0.8 5969.2 0.3 $\pm$ 0.4	[138] [55] [138]
Hypoxia-Induced Factor- α (μ l)	~0.75	~2.4	[216]
Serum AT1-AA (RLU, % over baseline)	~12	~70	[124]
AngII (fmol/L)	53.3 $\pm$ 10.1	30.15 $\pm$ 2.3	[217]
2-Methoxyestradiol (2-ME) ng/ml	~1.5	~0.8	[128]
ICAM (ng/mL)	169.01 $\pm$ 6.71	174.96 $\pm$ 14.35	[154]
VCAM (ng/mL)	307.90 $\pm$ 10.15	322.76 $\pm$ 12.62	[154]
Plasma Nitrite (μ mol/L)	8.7 $\pm$ 2.3 13.0 $\pm$ 4.3 ~40	5.2 $\pm$ 2.7 18.1 $\pm$ 6.2 ~50	[138] [218] [211]
L-arginine in Platelets	62.0 $\pm$ 31.1	19.2 $\pm$ 10.5	[138]
Serum ADMA (nmol/L)	~620	~750	[211]
ET-1 (pmol/L) Pg/ml	12 $\pm$ 1.0 3.6 $\pm$ 1.0	22.6 $\pm$ 2.0 5.1 $\pm$ 1.4	[146] [218]
6-keto PGF1 α (pg/mL)	254.51 $\pm$ 27.31	158.01 $\pm$ 15.20	[154]
TXB ₂ (pg/mL)	46.36 $\pm$ 4.23	41.28 $\pm$ 8.42	[154]
6-keto PGF1 α /TXB ₂ Ratio	7.21 $\pm$ 0.91	5.05 $\pm$ 0.55	[154]
EDHF in subcutaneous arteries (NO, PGL ₂ -independent relaxation to 3 μ M bradykinin %)	~65	~40	[172]
Serum MMP-2, MMP-9 (ng/ml) Serum TIMP-1, TIMP-2 (ng/ml)	669, 390 148, 228	834, 290 213, 232	[219]
Serum 25-OH vitamin D (ng/ml)	49.2 $\pm$ 29.2	18.21 $\pm$ 17.1	[58]
Serum Aldosterone (pg/ml)	5176 $\pm$ 417	3602 $\pm$ 514	[124]
Serum uric acid (mg/dl)	2.8	6.1	[55]

Table 3

Plasma and Vascular Tissue Levels, of Bioactive Factors and Vascular Mediators in Norm-Preg and HTN-Preg in Rodents

Marker	Normal Pregnancy	HTN-Preg	Ref
VEGF (pg/ml)	~9 830+/-33	~8 594+/-34	[104] [220]
PIGF (pg/ml)	~29 1.7+/-0.5	~27 0.28+/-0.05	[104] [220]
sFlt-1 (pg/ml)	774.91 ±26.81 82±26	1228.80±116.29 660±270	[104] [220]
sEng (ng/ml) (arbitrary pixel units, apu)	178.52±5.33 0.05±0.01	183.44±8.294 0.10±0.02	[104] [67]
TNF-α (pg/ml)	14.8±3.3	40±7.6	[83]
Plasma IL-6 (pg/ml)	287±12	778±29	[107]
Plasma IL-17 (pg/ml)	245 ± 45	714 ± 30	[107]
Placental ROS (Relative Light Unit, RLU)	436±4	702±5	[108]
O ₂ ^{•-} in placenta	1 ±0.03	1.63±0.19	[175]
Urinary isoprostane (pg/μg protein)	56.41	89.03	[82]
Hypoxia-Inducible Factor-α (HIF-α) In placenta (apu)	↑ 0.68±0.09	↑↑ 1.42±0.25	[120] [67]
Serum AT ₁ -AA (unit)	0.22 ±0.1 0.6±0.3U	13±0.7 15.3±1.6U	[126] [221]
Plasma NO (μM) Plasma L-arginine (μM)	11.20 ±1.05 10.47 ±2.31	9.87 ±0.59 5.08 ±0.81	[104] [104]
Urinary nitrite/nitrate (μmol/24 hr)	46.4±5.3	49.8±6.4, ↑, --, ↓	[66]
Plasma ET-1 (pg/ml)	1.7±0.3	2.2±0.3	[222]
PreproET-1 mRNA in renal cortex (unit)	37.7±2.4	29.4±2.8	[147]
PreproET-1 mRNA in renal medulla (unit)	24.0±2.6	44.1±6.3	[147]
HO-1 in Placenta In placenta (apu)	1 ±0.11 2.5±0.1	0.69 ± 0.02 1.4±0.3	[175] [67]
VSM [Ca ²⁺] _i (nM) Basal Phenylephrine (10 ⁻⁵ M) AngII(10 ⁻⁷ M)	63±5 149±8 149±8	109±8 234±1 225±9	[180] [180] [181]
Placental MMP-2 (Density units)	0.85±0.19	0.43±0.14	[77]
Placental MMP-9 (Density units))	0.24±0.07	0.11±0.04	[77]
Plasma aldosterone (pg/mL)	1377 ± 272	544 ± 92	[124]
Albumin/Creatinine (μg/mg)	132±17	242±27	[124]