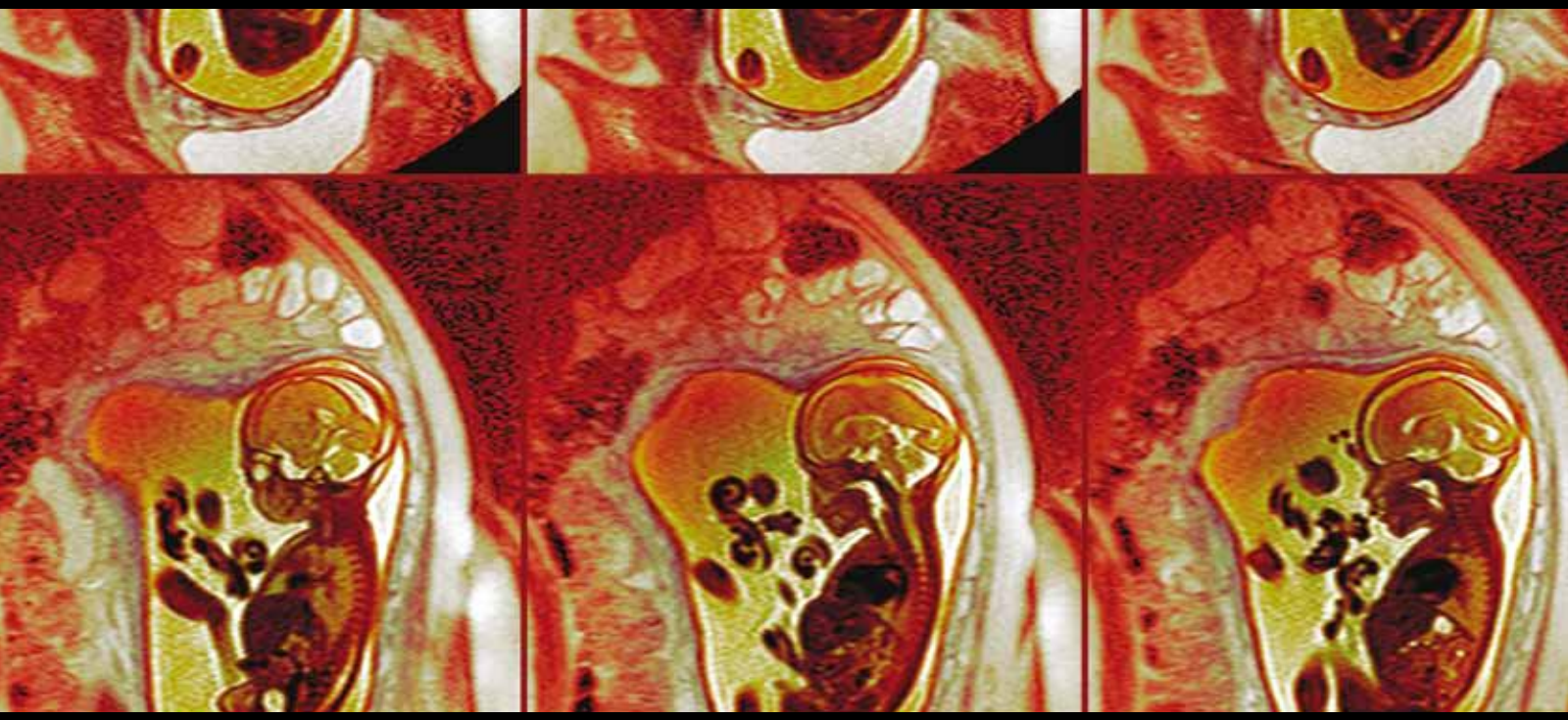
Journal of Pregnancy

Preeclampsia: Molecular Mechanisms, Predisposition, and Treatment

Guest Editors: Nikolaos Vitoratos, Nikolaos Vrachnis, Christos Iavazzo, and Maria Kyrgiou



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Editorial Board

Contents

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Editorial

Preeclampsia: Molecular Mechanisms, Predisposition, and Treatment

Nicolaos Vitoratos,[1] Nicolaos Vrachnis,[1] Christos Iavazzo,[1] and Maria Kyrgiou[2]

[1] *2nd Department of Obstetrics and Gynecology Aretaieio Hospital, University of Athens, 11528 Athens, Greece*
[2] *Department of Obstetrics and Gynaecology, Hammersmith Hospital, Queen Charlotte's and Chelsea Hospital, London W12 0HS, UK*

Correspondence should be addressed to Nicolaos Vitoratos, nikolasvitoratos@yahoo.gr

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Preeclampsia is a disorder characterised by vascular endothelial dysfunction and vasospasm that occurs after 20 weeks of gestation till 4–6 weeks postpartum. The global incidence of preeclampsia has been estimated at 2–14% of all pregnancies. Despite the advances made in the field, research is still organised to clarify the possible molecular mechanisms or predisposing factors of preeclampsia as well as the treatment options for such a significant disorder. Papers were selected on the basis of fundamental research ideas or reviews in the field. This special issue is comprising of seven papers which are focused on the better understanding of preeclapsia.

One of the papers deals with the correlation of preeclampsia with hypoxia, thrombosis, and inflammation. The authors suggest that there is no accurate test for predicting preeclampsia. The role of markers such as sFLT, sEng, products of fetal and placental origin, markers of renal or endothelial damage, or markers of oxidative stress is presented by acting as secondary pathways to the pathophysiological changes that precede the clinical onset of preeclampsia. A combination of such markers is proposed in order to increase the detection accuracy earlier in the pregnancy and hopefully allow for more effective prophylactic strategies.

Another paper sought to validate the use of urinary podocyte (podocyturia) as a single diagnostic marker in preeclampsia and in differentiating from other high-risk pregnancy states with similar presentations. The researchers discovered that podocyte loss is present not only in preeclampsia but in other high-risk pregnancy states. In addition, podocyturia was not found in a majority of patients diagnosed with preeclampsia. So, they realized that their findings had relatively low sensitivity and specificity, but they proposed further research regarding the predictive value of podocyturia in preeclampsia in larger studies.

The authors of one of the paper investigated whether there is an association between angiotensin converting enzyme (ACE) insertion/deletion (I/D) polymorphism and preeclampsia in 236 pregnant women. They showed that there was significant difference in terms of genotype distribution between preeclampsia and controls, while it was not found any difference in allele frequency for ACE I/D polymorphism. A possible reason for the inconsistency could be the genetic basis that caused different susceptibilities among different populations.

Moreover, one paper is mentioning that both women and children exposed to preeclampsia exhibit an adverse vascular phenotype, a propensity to subclinical atherosclerosis, and increased risk of adverse cardiac and vascular events in future life. They suggest that further studies into the mechanisms such as vascular dysfunction underlying the altered cardiovascular phenotype might provide unique insight into pathophysiological or molecular links between preeclampsia and cardiovascular disease which may direct us to novel treatment strategies for both conditions. Improvement in vascular function is also proposed as a valuable intermediate end point in studies aiming to reduce risk in this potentially young and generally asymptomatic population before the onset of clinical disease.

F. J. Valenzuela and colleagues have recently reviewed some polymorphisms in important candidate genes involved in different pathogenic mechanisms related to preeclampsia

and concluded that various studies in different populations have identified maternal polymorphisms associated with preeclampsia through candidate gene approaches.

Luizon and colleagues with a Letter to the Editor add to the paper of F. J. Valenzuela et al. by further referring to candidate genes related to angiogenesis and endothelial dysfunction in preclampsia performed in the Brazilian population. Specifically, genotypes and haplotypes formed by polymorphisms of VEGF, eNOS, and MMP-9, along with an example of the interaction among these genes in the prediction of preeclampsia provide additional information with clinical relevance to its susceptibility.

An additional paper is a review of the molecular mechanisms which are contributing to the pathogenesis of preeclampsia. Altered angiogenic balance, systemic inflammation, dysregulation of Renin-Angiotensin system, and placental hypoxia or ischemia are mechanisms leading to the pathogenesis of preeclampsia. However, it is unknown whether the mechanisms act independently or have synergistic effects.

Acknowledgment

The Guest Editors recommended papers for publication on the basis of academic merit and would like to thank all the authors of this special issue for contributing the high-quality papers. We would also like to thank the referees who have critically evaluated the papers within the short stipulated time. Finally, we hope the readers will share our enthusiasm, and find this special issue very useful.

Nicolaos Vitoratos
Nicolaos Vrachnis
Christos Iavazzo
Maria Kyrgiou

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Letter to the Editor

Polymorphisms and Haplotypes in Candidate Genes Related to Angiogenesis and Endothelial Dysfunction in Preeclampsia

Marcelo Rizzatti Luizon,[1] Ana Carolina Taveiros Palei,[2] and Valeria Cristina Sandrim[3]

[1] *Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Avenida Bandeirantes, 3900, 14049-900 Ribeirao Preto, SP, Brazil*
[2] *Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas, 13081-971 Campinas, SP, Brazil*
[3] *Núcleo de Pós-Graduação e Pesquisa, Santa Casa de Belo Horizonte, Rua Domingos Vieira 590, 30150-240 Belo Horizonte, MG, Brazil*

Correspondence should be addressed to Valeria Cristina Sandrim, valsandrim@yahoo.com.br

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Valenzuela and colleagues have recently reviewed some polymorphisms in important candidate genes involved in different pathogenic mechanisms related to preeclampsia (PE) and concluded that various studies in different populations have identified maternal polymorphisms associated with PE. However, we would like to contribute to some studies regarding candidate genes related to angiogenesis and endothelial dysfunction in PE performed in the Brazilian population. Specifically, genotypes and haplotypes formed by polymorphisms of *VEGF*, *eNOS* and *MMP-9*, along with an example of the interaction among these genes in the prediction of PE. Our suggestions may provide additional information with clinical relevance to PE susceptibility.

Valenzuela et al. [1] have recently published an interesting *Review Article* in a special issue of *Journal of Pregnancy* discussing some polymorphisms in important candidate genes involved in different pathogenic mechanisms related to preeclampsia (PE). They have concluded that various studies in different populations have identified maternal polymorphisms associated with PE through candidate gene approaches [1]. However, some important references from studies performed in the Brazilian population were not cited, more specifically regarding the mentioned candidate genes related to *vascular and endothelial function*.

For example, our group has recently demonstrated a main effect of vascular endothelial growth factor (VEGF) genotypes and haplotypes involving three clinically relevant single nucleotide polymorphisms (SNPs) localized in the promoter region of *VEGF*; −2578C/A (rs699947), −1154G/A (rs1570360), and −634G/C (rs2010963) in the development of PE, but not with gestational hypertension (GH) [2]. When white and nonwhite pregnant women were considered together, no significant differences were found in the distributions of *VEGF* genotypes or haplotypes ($P > 0.05$). However, significant differences were found in genotypes distributions for two *VEGF* polymorphisms (−2578C/A and −634G/C; both $P < 0.05$) between the healthy pregnant (HP) and the PE groups when only white subjects were considered in the analysis [2]. Importantly, the haplotype including the alleles −2578C, −1154G, and −634C, which is associated with higher VEGF gene expression elsewhere [3], was less common in the PE group compared with the HP group ($P = 0.0047$ [2]). Moreover, we have previously reported marked interethnic differences in the distribution of these *VEGF* genotypes and haplotypes [4]. These differences could explain why we have found significant associations between *VEGF* genotypes and one *VEGF* haplotype with preeclampsia when only white women were considered in the analysis [2].

Regarding the endothelial nitric oxide synthase (eNOS), our group had previously examined the association of three clinically relevant polymorphisms in the promoter region (−786T/C, rs2070744), in intron 4 (a variable number of tandem repeats, VNTR) and in exon 7 (Glu298Asp, rs1799983) of *eNOS* with PE and GH [5]. No differences were observed in the frequencies of genotypes and alleles of the three polymorphisms among PE, GH, and HP groups (all $P > 0.05$). However, the haplotype "T Glu a" was more common in HP than in GH or PE (20 versus 6 and 6%, resp.;

$P < 0.0032$). Conversely, the haplotype "C Glu a" was more common in GH and PE than in HP (17 and 17 versus 5%; $P = 0.0061$). These findings suggest a contribution of *eNOS* haplotypes to the development of hypertensive disorders of pregnancy (HDP) that is obscured when specific *eNOS* genotypes alone are considered [5].

In addition, we have also examined whether *eNOS* polymorphisms and haplotypes affect the responsiveness to antihypertensive therapy in women with GH or PE [6]. Although we found no significant differences in genotype or allele distributions when responsive and nonresponsive groups were compared (both PE and GH; all $P > 0.05$), *eNOS* haplotype distribution differed in PE (but not in GH) responsive and nonresponsive groups ($P = 0.0003$), thus suggesting that eNOS haplotypes affect the responsiveness to antihypertensive therapy in PE [6].

Once PE is associated with decreased nitric oxide (NO) formation and no previous study had examined whether *eNOS* polymorphisms affect this alteration, we hypothesized that NO bioavailability may be modulated by *eNOS* polymorphisms in pregnancy [7]. No effects in nitrite concentrations were found among PE women with different *eNOS* genotypes and haplotypes ($P > 0.05$). However, the "C Glu b" haplotype, which was more frequent in the HP group than in the PE group (20 versus 5; $P = 0.0044$), was associated with higher nitrite concentrations than the other haplotypes in HP ($P < 0.05$). These findings indicate that *eNOS* polymorphisms affect endogenous NO formation in normal pregnancy, but not in PE and that the "C Glu b" haplotype may protect against the development of PE by increasing endogenous NO formation [7].

We would like to contribute to the *Review Article* of Valenzuela et al. [1] with our data about another candidate gene related with angiogenesis. Abnormal production of matrix metalloproteinases (MMPs), especially MMP-9, may also play a role in HDP [8, 9]. These alterations may result from functional polymorphisms in the promoter region of *MMP-9* gene, which are known to change MMP-9 expression. Therefore, we examined whether the polymorphisms −1562C/T (rs3918242) and $-90(CA)_{13\text{-}25}$ (rs2234681) in the promoter of *MMP-9* were associated with HDP [8, 9]. The CT genotype and T allele for the −1562C/T polymorphism, besides the haplotype including the alleles T and H, were more commonly found in GH, but not in PE, compared with the HP group (both $P < 0.05$), suggesting that *MMP-9* polymorphisms may be associated with GH, but not with PE [8, 9]. In addition, the GH patients with the LH genotype for the $-90(CA)_{13\text{-}25}$ polymorphism have higher plasma MMP-9 concentrations than those with other genotypes [8]. Furthermore, we have examined whether MMP-9 polymorphisms affect the responsiveness to antihypertensive therapy in women with GH or PE [8]. The T allele for the −1562C/T polymorphism and the haplotype combining T and H alleles were associated with lack of responsiveness to the antihypertensive therapy in GH, and the haplotype combining C and H alleles was associated with lack of responsiveness to the antihypertensive therapy in PE [8].

Valenzuela et al. [1] have also pointed out that the findings from candidate gene polymorphisms will need to be complemented by evaluation of interaction between genes. We have recently provided an example of epistasis in PE considering the *MMP-9* and *VEGF* polymorphisms [10]. The results from single locus analysis showed significant differences in the distribution of genotypes and alleles for the *VEGF* −634G/C polymorphism when PE was compared to HP and for the *MMP-9* −1562C/T polymorphism when GH was compared to HP, respectively (all $P < 0.05$). These results are in agreement with our previous findings [2, 9]. However, we have observed a significant interaction between *MMP-9* and *VEGF* genes associated with the PE group compared to HP [10]. The interaction between *MMP-9* and *VEGF* polymorphisms associated with the PE group is obscured when specific genotypes of these single genes are considered, thus highlighting the importance of gene-gene interactions as major determinants to complex diseases, including PE [11].

In conclusion, we consider that the present may contribute to the interesting review article of Valenzuela et al. [1]. The findings and suggestions from our studies on candidate genes to PE may contribute with additional information of clinical relevance to PE susceptibility.

Conflict of Interests

The authors declare no conflict of interests.

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Research Article

Studies on Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism and Genotype Distributions in Turkish Preeclampsia Patients

Ceyhun Bereketoğlu, Mülkiye Kasap, and Ayfer Pazarbaşı

Department of Medical Biology and Genetics, Faculty of Medicine, Cukurova University, 01330 Adana, Turkey

Correspondence should be addressed to Ayfer Pazarbaşı, payfer@cu.edu.tr

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Placental, immune and genetic factors are thought to play an important role in preeclampia (PE)'s pathophysiology. Angiotensin-Converting Enzyme (ACE) plays a vital role in the renin-angiotensin-system (RAS) which regulates blood pressure by converting angiotensin I into a powerfull vasoconstrictor angiotensin II. A deletion polymorphism (D allele) has been reported to be associated with elevated ACE activity. The aim of the this study was to investigate whether there is an association between angiotensin converting enzyme (ACE) insertion/deletion (I/D) polymorphism and PE. In this study, 120 preeclamptic and 116 normotensive Turkish pregnant women were genotyped for ACE I/D polymorphism and the distribution of genotype and allele frequencies of this polymorphism in preeclampsia and controls were evaluated. Codominant, dominant and recessive models were appplied in ACE gene I/D polymorphism. In the codominant model, DD genotype was found significantly more frequent in preeclampsia than controls ($P = 0.016$). Moreover, in dominant model (DD frequency versus DI+II frequency) there was a significant relation between DD genotype and preeclampsia ($P = 0.006$). D allele frequency was 64.6% in preeclampsia while it was 56.1% in controls ($P = 0.062$). In conclusion, there was significant difference in genotype distribution between preeclampsia and controls.

1. Introduction

Preeclampsia (PE) is a disorder that occurs in women with a new-onset of hypertension and proteinuria after 20 weeks of pregnancy. It plays an important role in perinatal mortality and morbidity, as well as maternal mortality [1–8]. It affects 3–5% of all pregnancies worldwide, and the best treatment is delivery [4, 5, 9]. Although the aetiology of preeclampsia is still unclear, there are some evidences that preeclampsia is associated with abnormal placentation which is related to poor maternal defense mechanisms and impaired placentation in early gestation resulted from low-resistance uteroplacental circulation [10–12].

Angiotensin-converting enzyme (ACE, EC 3.4.15.1, a peptidyl carboxypeptidase) plays a vital role in the rennin angiotensin system (RAS) which regulates blood pressure by converting angiotensin I into a powerful vasoconstrictor angiotensin II. High ACE activity can contribute to hypertension because of its vasoconstriction effect [13, 14]. An insertion/deletion (I/D) polymorphism in the ACE gene occurs due to the insertion or deletion of an Alu 289 base pairs (bp) sequence located at intron 16 [15]. A deletion polymorphism (D allele) has been reported to be associated with elevated ACE activity [16]. Some investigators have reported in women from various geographical origins an association between the ACE D allele or DD genotype and increased risk of preeclampsia or pregnancy-induced hypertension [15–19], whereas others could not [20–23]. Women included in this study were all Caucasian.

The aim of the study was to investigate whether there is an association between ACE intron 16 I/D polymorphisms and PE.

Table 1: The clinical characteristics of the study population and controls.

	Mean of maternal age (years)	Mean of maternal weight (kg)	Number of pregnancy	Gestational age (weeks)	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)
PE	29,00 ± 7,044	78,89 ± 10,201	2,29 ± 1,597	34,615 ± 4,698	151,56 ± 16,116	98,85 ± 12,934
Controls	27,41 ± 5,317	77,01 ± 9,985	2,27 ± 1,639	37,060 ± 4,095	110,54 ± 10,000	71,63 ± 8,044

2. Materials and Methods

Written approval was obtained from the Ethics Committee of Cukurova University Hospital and Baskent University, and all patients gave their informed consent before peripheral blood samples were taken. Information was enrolled retrospectively about one hundred twenty (120) preeclamptic women and one hundred fourteen (114) normotensive women with no history preeclampsia who delivered at two university hospitals located in Adana (Cukurova and Baskent University Hospitals) between September 2009 and August 2010. PE cases included both severe and mild PE, and all of them were early-onset cases (after 20 weeks). A total of 234 patients were studied. Clinical characteristics of the study population are reported in Table 1.

Preeclampsia was defined using the criteria of the National High Blood Pressure Education Program Working Group: (1) increase of 30 mm Hg or greater of systolic blood pressure, (2) increase of 15 mm Hg or greater of diastolic blood pressure (both criteria 1 and 2 refer to before and after 20 weeks of gestation), (3) if previous blood pressure was not known, a blood pressure must be ≥140 mm Hg for systolic and ≥110 mm Hg for diastolic after 20 weeks of gestation, (4) in addition to blood pressure, proteinuria was defined as the excretion of 0.3 g/L (1+ on a dipstick) or greater [24]. Women with a significant past medical history such as diabetes, chronic hypertension, pregnancies with malformed fetuses or infections, twin pregnancies were excluded from the preeclampsia patients and controls. The controls were normotensive women who had no history of preeclampsia and were recruited from the same centers randomly.

Maternal DNA was isolated from peripheral venous blood leukocytes using standard salting out method as previously described [25]. ACE intron 16 I/D polymorphism was genotyped by 2 PCRs using 3 primers [26]. The primers of first PCR were 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ and 5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′. The 190 and 490 bp products of this PCR were from D and I alleles, respectively. The first amplification reaction was carried out in a total volume of 25 μL, using 200 ng genomic DNA, 25 mM dNTPs, 10 pmol of each primer, 1 U Taq DNA polymerase (Vivantis), and 2.5 μL ViBuffer S (Vivantis). PCR conditions were 94°C for 5 minutes, followed by 30 cycles at 94°C for 1 minute, 58°C for 1 minute, 72°C for 2 minutes, and a final step at 72°C for 4 minutes.

To avoid the misidentification of DI genotypes as DD, a second PCR was performed with the same antisense primer as in the first and different sense primer as 5-TTT GAG ACG GAG TCT CGC TC-3′ that generates a 408 bp fragment only in the presence of the I allele. The second PCR was carried out in a total volume of 25 μL with the same reagents of the first amplification reaction. PCR conditions were 94°C for 5 minutes, followed by 40 cycles at 94°C for 1 minute, 60°C for 75 seconds, 72°C for 1 minutes, and a final step at 72°C for 10 minutes.

Table 2: Angiotensin-converting enzyme (ACE) polymorphism in normal pregnancies and in pregnancies complicated by preeclampsia (PE).

Model	Controls $n = 114$ Distribution	PE $n = 120$ Distribution	P value
Codominant			
DD	30 (26,3%)	52 (43,3%)	0.016
DI	68 (59,6%)	51 (42,5%)	
II	16 (14,0%)	17 (14,2%)	
Recessive			
II	16 (14,0%)	17 (14,2%)	0.977
DD+DI	98 (86,0%)	103 (85,8%)	
Dominant			
DI+II	84 (73,7%)	68 (56,7%)	0.006
DD	30 (26,3%)	52 (43,3%)	
Allele			
D	128 (56,1%)	155 (64,6%)	0.062
I	100 (43,9%)	85 (35,4%)	

PCR products were analysed by 2% agarose gel after staining by ethidium bromide. Statistical analyses were carried out with the SPSS version 15.0. Pearson's chi-squared test was used for the statistical evaluation of the individual allele and genotype frequencies. The level of statistical significance was defined as $P < 0.05$. In the ACE I/D polymorphism, data were analyzed under three models: a codominant, a dominant, and a recessive model.

3. Results

The clinical characteristics of the study population are shown in Table 1. Mean maternal age was similar between controls and preeclampsia, while gestational age was significantly higher in controls than preeclampsia.

The genotype distributions and allele frequencies for ACE I/D polymorphism were summarized in Table 2. For ACE I/D polymorphism, the frequency DD genotype was 43.3% in preeclampsia, while it was 26.3% in controls analyzing data under a codominant model. In this model, the difference was found statistically significant ($P = 0.016$). Moreover, in the dominant model (DD frequency versus DI+II frequency), the difference between the two groups was found

statistically significant ($P = 0.006$). The ACE D allele frequency was 64.6% in preeclampsia and 56.1% in controls, and the difference was not found statistically difference ($P = 0.062$).

4. Discussion

The present study showed an association between ACE DD genotype and preeclampsia in Turkish population. In the analyzed Turkish population, PE cases included both severe and mild PE and all of them were early-onset cases (after 20 weeks). On average, deliveries occurred about 3 weeks earlier in the preeclamptic women than in the controls.

The results of previous studies on association of ACE I/D polymorphism with preeclampsia were conflicting presumably attributable to differences in study population, genetic backgrounds, and size of study groups. Some studies showed significantly higher incidence of DD genotype and/or D allele in preeclampsia and/or pregnancy-induced hypertension [15–19]. On the other hand, no difference in genotype distribution and allele frequency and no association between DD genotype and occurrence of preeclampsia were found in some studies [20–23]. A possible reason for the inconsistency among these reports may be a genetic basis that causes different susceptibilities among different populations.

In the present study, for ACE I/D polymorphism analysis, we used three models: codominant, recessive, and dominant models which were used in a study on Caucasian population [15]. We detected differences in genotype distribution between preeclampsia and controls for the ACE gene polymorphism when data were evaluated using a dominant model, considering DD frequency versus DI+II and using a codominant model. In both models, we found a higher incidence of DD genotype in preeclampsia when compared to controls. However, in the previous study on caucasian population, a higher difference was found when data were evaluated using a recessive model, considering II frequency versus DI+DD frequency [15]. Moreover, we found no association between allelic frequency and preeclampsia. A weakness of our study is that the data observed was obtained from a limited population (Turkish women). The main limitation of the study is the lack of the circulating cytokine measures that are needed to prove a functional relationship between polymorphisms, elevated cytokines, and PE.

In conclusion, although our results show an association between the ACE DD genotype and preeclampsia in the analyzed Turkish population, further studies using a larger number of subjects and analyses that include genetic, environmental, and other potential factors are needed to confirm these results.

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Review Article

Molecular Mechanisms of Preeclampsia

N. Vitoratos, D. Hassiakos, and C. Iavazzo

2nd Department of Obstetrics and Gynecology, University of Athens, Aretaieion Hospital, 11528 Athens, Greece

Correspondence should be addressed to C. Iavazzo, christosiavazzo@hotmail.com

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Preeclampsia is one of the leading causes of maternal morbidity/mortality. The pathogenesis of preeclampsia is still under investigation. The aim of this paper is to present the molecular mechanisms implicating in the pathway leading to preeclampsia.

1. Introduction

Preeclampsia is one of the leading causes of maternal morbidity/mortality and preterm delivery worldwide [1]. It is a syndrome defined by the onset of hypertension (≥140/≥90 mmHg) and proteinuria (≥0,3 gr/24 h) after 20 weeks of gestation in a previously normotensive woman that also may be associated with myriad, other signs and symptoms, and often with subnormal fetal growth [2, 3]. Most commonly, preeclampsia occurs in healthy nulliparous women. However, multiparous pregnant women with a new partner have an increased risk of preeclampsia similar to that of nulliparous women [4]. Women with a history of preeclampsia in a prior pregnancy are at increased risk of developing preeclampsia in future pregnancies [5]. A history of preeclampsia in the father's mother also confers an increased risk [6]. Several medical conditions, such as chronic hypertension, diabetes mellitus, renal disease, and hypercoagulable states are associated with increased preeclampsia risk [7, 8]. Additionally, obstetrical conditions with increased placental mass increase the risk of preeclampsia. These include hydatidiform mole [9] and multifetal gestation [10].

Delivery of the placenta remains the only known treatment for this clinical disease, suggesting that the placenta is the principal contributor to the pathogenesis of preeclampsia. It is well known that the first step for the development of preeclampsia is the inadequate placental cytotrophoblast invasion, impaired trophoblast invasion, and inadequate maternal spiral artery remodeling which results in placental ischemia and hypoxia. However, placental ischemia does not always generate the clinical symptoms of preeclampsia. Many molecular mechanisms are contributed to the pathogenesis of preeclampsia. Altered angiogenic balance, systemic inflammation, dystregulation of renin-angiotensin system, and placental hypoxia and ischemia are mechanisms which contribute to the pathogenesis of pre-eclampsia, although it is unknown whether the mechanisms act independently or have synergistic effects.

2. Altered Angiogenic Balance

2.1. Angiogenic Factors. A variety of angiogenic factors are produced from the human placenta. The most important between them are the vascular endothelial growth factor (VEGF) and the placental growth factor (PIGF) [11]. VEGF is an endothelial-specific mitogen that plays a key role in promoting angiogenesis. VEGF stabilizes endothelium in mature blood vessels [12]. VEGF'S activities are mediated primarily by its interaction with two high-affinity receptors tyrosine kinases—kinase-insert domain region (KDR or VEGF R-2) and fms-like tyrosine kinase-1 or flt-1. Both receptors are expressed on vascular endothelial cell surface [13]. PIGF is also an angiogenic growth factor that is thought to amplify VEGF signalling by displacing VEGF from the flt-1 receptor and allowing it to bind to the more active kinase-insert domain (KDT) receptor [14, 15].

Recent research has shown that soluble flt-1 is released by the placenta into the maternal circulation and, that is, contributes to the hypertension, proteinuria, and endothelial cell dysfunction associated with preeclampsia [16]. sflt-1 antagonizes both VEGF and PIGF by binding them in

the circulation and preventing interaction with their endogenous receptors [17, 18]. New variants of sflt-1 have been discovered such as sflt1–14, which is also a potent VEGF inhibitor [19, 20]. The level of SFLt-1 in the plasma of women with preeclampsia is elevated and that of VEGF is diminished in comparison with that of women with complicated pregnancies [21]. Furthermore, administration of sflt-1 to rats resulted in elevated blood pressure and proteinuria, indicating that excessive placenta-derived sflt-1 max contributes to preeclampsia [21].

Factors responsible for excessive production of self-1 in preeclampsia have not been identified. However, recently it has been found that angiotensin II type 1 (AT) receptor autoantibodies which occur in women with preeclampsia contribute to increased production of sflt-1. Thus, IgG from women with preeclampsia stimulates the synthesis and secretion of sflt-1, via AT_1 receptor activation in human placental villous explants and human trophoblast cells. Another factor which contributes to increased production of sflt-1 is the hypoxic placenta [22]. Under other pathophysiological conditions such as cancer and hypoxia which generally stimulates angiogenic signalling, it remains poorly understood why hypoxic placenta produces the molecules that suppress angiogenesis in preeclampsia [23].

Soluble endoglin is another antiangiogenic protein, which acts to get it with sflt-1 to induce a severe preeclampsia-like syndrome in pregnant rats. Circulating soluble endoglin levels increased markedly beginning from 2 to 3 months before the onset of preeclampsia. An increased level of soluble endoglin was usually accompanied by an increased ratio of sflt-1 [24].

Experiments data have shown that VEGF stimulates the production of both nitric oxide (NO) and PGI_2 [25]. On the other hand, a high concentration of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase, has been found in preeclamptic women [26].

Women with bilateral notches who later developed preeclampsia had a striking elevation in the concentration of the NO synthase inhibitor [27].

ADMA is normally metabolized to citrulise through the action of dimethylarginine-dimethylaminohydrolase I, II (DDAH I, II). Oxidative stress seen in preeclampsia diminishes the action of the alone enzymes leading to high concentration of ADMA [27].

3. The Role of Relaxin in Preeclampsia

Relaxin is produced by the corpus luteus of the ovary and rises early in pregnancy, and chorionic gonadotropin produced by the placenta is a major stimulus for relaxin secretion during pregnancy.

Relaxin has renal vasodilatory effect [28], and it also diminishes the relaxin vasoconstrictor response to angiotensin II. Moreover, reduced myogenic reactivity of small renal arteries is observed after relaxin administration [29, 30]. Recently, it has been proposed that relaxin via relaxin receptor upregulates vascular gelatinase activity during pregnancy, contributing to renal vasodilation through activation of endothelial endothelin B (ET_B) receptor which activates nitric oxide synthase III and the production of NO [31]. Thus, increased vascular gelatinase activity by relaxin is thought to be a proximal step in the vasodilatory pathway of pregnancy [32].

Circulating levels of immunoreactive relaxin have been reported to be similar in women with preeclampsia and normal pregnancy [33]. However, whether circulating relaxin bioactivity may be deficient during the disease is uncertain [34]. Furthermore, mutations or polymorphisms of the ET_B receptor or of endothelial NO synthase that reduce activity may predispose a woman to preeclampsia by impairing trophoblast invasion on the one hand or by compromising maternal endothelial behaviour on the other [35, 36].

4. Inflammatory Cytokines in the Pathophysiology of Preeclampsia

Reduced uterine perfusion during pregnancy is an important initialling event in preeclampsia. Inflammatory cytokines are thought to link placental ischemia with cardiovascular and renal dysfunction [37]. In normal pregnancy TNF-α is low in the first trimester and subsequently increases with advancing gestation age [38]. Some studies report higher TNF-α levels in women with established preeclampsia [39, 40]. Increased levels of TNF-α antigen and mRNA have been described in placental tissue from preeclamptic women [41].

Because TNF-α may impair insulin signalling, inhibit lipoprotein lipase, induce PAI-1, and directly contribute to endothelial dysfunction, this cytokine may be involved in the pathogenesis of preeclampsia [42].

There are also findings showing that chronic infusion of IL-6 into normal pregnant rats stimulates the renin-angiotensin system (RAS) [37].

Natural killer (NK) cells, dendritic cells, and macrophages are mediators of innate immunity. Macrophages and dendritic cells are the major antigen-presenting cells in the uterus, and they facilitate adaptation of the immune response to prevent rejection of the embryo [43].

Several studies have found a statistically significant increase in macrophages and dendritic cells in preeclamptic placentas compared to placentas from normotensive pregnancies [43, 44]. An increase in the concentration of cytokines, molecules capable of recruiting macrophages, and dentritic cells has also been found in preeclamptic placentas [44].

The increased presence of cytokines, macrophages, and dendritic cells in preeclamptic placentas supports the hypothesis that an inflammatory milieu presents in women with preeclampsia [44].

5. Activation of Renin-Angiotensin System (RAS)

Renin-angiotensin system is one that controls blood pressure [45]. The expression of rennin mRNA was detected in human deciduas, macrophages, chorioamniotic membrane, and vascular smooth muscle cells [46].

Angiotensin II receptor type I (AT_1) was shown to be localized both in villous and extravillus trophoblasts, and this AT_1 responds to exogenously administrated angiotensin II [47].

The circulating level of angiotensin II increases as the pregnancy advances [48]. In preeclampsia, the circulating level of angiotensin is rather decreased [49], despite the fact that the vascular sensitivity to angiotensin is elevated in hypertensive pregnant women.

The AT_1 receptor gene expression was higher in placenta than in deciduas for both normal and preeclamptic women. However, the deciduas of preeclamptic women has a significantly higher AT_1 receptor gene expression than normal pregnant women [50]. It has been found that the gene encoding the AT_1 receptor was upregulated in the deciduas of preeclamptic women but not in normal control [51]. Circulating agonistic autoantibodies directed at the angiotensin II type 1 receptor (AT_1-AA_s) have been discovered in women with preeclampsia [52].

Thus the increased decidual AT_1 expression in preeclampsia may be the initial step for a profound RAS activation. Furthermore, the presence of AT_1-AA_s is able to activate cells via the AT_1 receptor and initiate signaling events that could contribute to development of preeclampsia. Thus, release of soluble flt-1 can be triggered by angiotensin II stimulation, raising an imbalance between angiogenic vascular endothelial growth factors and antiangiogenic soluble factors [21]. Zhou et al. [21] have shown that the inhibition of AT_1 administration of losartan or FK506 resulted in reduced SVEGFR-1. Thus, maternal SVEGFR-1 can be elevated not only by poor placentation but also by AT_1 activation in which angiotensin II and AT_1-AA_s are potentially implicated.

6. Placental Hypoxia and Ischemia

Impaired trophoblast invasion and inadequate maternal spiral artery remodelling result in placental ischemia and hypoxia. It is unknown, however, whether abnormal placentation leads to systemic vascular dysfunction and the appearance of preeclampsia.

Defective trophoblast invasion and inadequate maternal spiral remodelling frequently result in intrauterine growth restriction or other complications of pregnancy (preterm labor e.g.) without preeclampsia even to normal full-term pregnancy [53]. Women living in high altitudes have an increased risk of developing preeclampsia [54], while cigarette smoking is associated with a reduced risk for preeclampsia [55]. Experiments in animals suggest that placental hypoxia contributes to preeclampsia by upregulating soluble antiangiogenic factors, inflammatory cytokines, downregulating angiogenic, and vasodilator factors [56].

Furthermore, in pregnant mice, an absence of 2-methoxyestradiol (2-ME), a natural metabolite of estradiol, results in a deficient of catechol-o-methyltransferase (COMT). These animals showed a preeclampsia-like phenotype [57]. The addition of 2-ME was shown to improve preeclampsia and suppress placental hypoxia and sflt-1 expression [57]. It is, however, unclear whether or not decreased COMT is the cause of the consequence of impaired placentation.

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Research Article

Podocyturia as a Diagnostic Marker for Preeclampsia amongst High-Risk Pregnant Patients

Belinda Jim,[1] Pascale Jean-Louis,[1,2] Andi Qipo,[1] David Garry,[2] Samia Mian,[3] Tulio Matos,[4] Christopher Provenzano,[1] and Anjali Acharya[1]

[1] *Division of Nephrology, Department of Medicine, Jacobi Medical Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA*
[2] *Department of Obstetrics and Gynecology, Montefiore Medical Center, Bronx, NY, USA*
[3] *Advocate Christ Medical Center, Oak Lawn, IL, USA*
[4] *Columbia University Medical Center, New York, NY, USA*

Correspondence should be addressed to Belinda Jim, belinda_bun_jim@yahoo.com

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Urinary podocyte (podocyturia) has been studied as a diagnostic marker for preeclampsia. We sought to validate its use in preeclampsia and in differentiating it from other high risk pregnancy states. We studied an obstetric population at high risk to develop preeclampsia (study group) and uncomplicated pregnancies (control group) by analyzing their urine sediment for podocytes within 24 hours of delivery. Podocytes were identified by immunohistochemistry using the podocyte-specific protein synaptopodin. Of the 56 patients who were enrolled, 29 patients were diagnosed with preeclampsia, 9 patients had hypertensive conditions such as chronic and gestational hypertension, 6 patients had Type I/II and gestational diabetes mellitus, 3 patients were classified as others, and 9 patients exhibited uncomplicated pregnancies. Podocyturia was identified in 11 out of 18 (38%) of patients with preeclampsia, 3 out of 9 (33%) with gestational and chronic hypertension, and 3 out of 6 (50%) with Type I/II and gestational diabetes mellitus. None of the 9 patients (0%) with uncomplicated pregnancies demonstrated podocyturia. The sensitivity and specificity of podocyturia for preeclampsia were found to be 38% and 70%. Our study showed that podocyturia does not appear to be a sensitive nor a specific marker to diagnose preeclampsia.

1. Introduction

Preeclampsia is a disorder affecting 5 to 10% of pregnancies and is clinically characterized by new-onset hypertension and proteinuria. Despite significant progress in our understanding of preeclampsia, there is a need for a reliable diagnostic biomarker for use in clinical practice. In the search for a biologically plausible biomarker, there have been attempts to reconcile clinical findings with pathologic changes in renal biopsies of preeclampsia. For example, renal pathology of preeclampsia in the kidney is classically described as "endotheliosis", or swelling of endothelial cells in the glomerulus. The podocyte, a specialized visceral epithelial cell that lines and forms the slit diaphragm of the glomerular basement membrane, has traditionally been thought to be unaffected. However, more recent microscopic studies demonstrate that podocytes are structurally changed and harbor protein resorption droplets [1]. Furthermore, there is evidence that selected podocyte-specific proteins such as nephrin, synaptopodin, and GLEPP-1 are downregulated in preeclampsia, while VEGF and Flt-1 are increased [2, 3]. Garovic et al. studied the use of podocytes in the urine (podocyturia) as diagnostic markers and found podocyturia to be highly sensitive and specific for preeclampsia [4]. We sought to study the use of podocyturia to diagnose preeclampsia and differentiate it from other conditions that may have a similar presentation in a high risk pregnancy population. We discovered that podocyturia was not very sensitive nor specific in making this diagnosis. Furthermore, podocyturia was found frequently in other high risk pregnancy states such as chronic hypertension and gestational diabetes.

2. Materials and Methods

2.1. Study and Control Subjects. We recruited two groups of patients all ≥18 years of age: uncomplicated pregnant subjects (control group) and women at risk for pregnancy complications (high-risk group) as described below from the obstetric inpatient service at Jacobi Medical Center, Bronx, NY, USA. Random urine samples were obtained from the subjects within 24 hours of delivery. Inclusion criteria for high-risk group were diagnosis of preeclampsia, chronic hypertension (HTN), gestational HTN, Type I and Type II diabetes mellitus (DM), gestational DM, mixed connective tissue disease, and pregnancies with fetal chromosomal abnormalities. Exclusion criteria were patients under the age of 18 and absence of the above-mentioned diagnosis. Inclusion criteria for the control group were uncomplicated pregnancies and deliveries and absence of the above-mentioned high risk pregnancy states. Exclusion criteria for the control group were <18 years of age, preexisting high risk pregnancy states or complicated deliveries. Diagnosis of preeclampsia fulfilled the criteria of new onset of HTN with blood pressure of 140/90 mmHg after 20 weeks of gestation and proteinuria of >300 mg of protein in a 24-hour urine specimen or 1+ protein on a urinalysis sample without evidence of another cause, such as urinary tract infection or inflammation. Chronic HTN was defined as preexisting HTN or blood pressure of 140/90 mmHg before 20 weeks of gestation. Gestational DM was defined as any degree of glucose intolerance with onset of first recognition during pregnancy. This study was approved by the Internal Review Board of Albert Einstein College of Medicine.

2.2. Urinary Protein Quantification. Urinary protein was quantified either from a 24-hour urine sample collection or extrapolated from a random urine dipstick. For example, when only a dipstick urine protein was available, the value was extrapolated to a 24-hour value based on the following: negative protein corresponds to less than 150 mg/24 hours, trace protein corresponds to 150 mg/24 hours, 1+ corresponds to about 200–500 mg/24 hours, 2+ to 0.5–1.5 g/24 hours, and 3+ to 2–5 g/24 hours.

2.3. Podocyturia. Twenty mL of freshly voided urine was centrifuged at 700 g for 5 min. The sediment pellet was carefully recovered by aspirating the supernatant, washed twice with PBS and resuspended in 1 mL of PBS. Aliquots of 100 μL of the resuspended sediment were centrifuged onto slides using the Shandon Cytospin 4 Cytocentrifuge (Thermo Electron Corporation, Asheville, NC), air-dried and fixed with 1 : 1 acetone/methanol for 10 minutes. The slides were immersed with PBS/1% H_2O_2 for 15 minutes and washed with deionized water. Subsequently, antigen retrieval was achieved by steam-heating in a solution of citrate buffer, pH 6.0, for 15 minutes and blocked with 10% horse serum in PBS and 2% BSA. Slides were incubated overnight with monoclonal mouse antihuman synaptopodin antibody at 1 : 1 dilution (gift of Dr. Peter Mundel, Massachusetts General Hospital, Boston, MA) followed by horse anti-mouse IgG at 1 : 1000 dilution (Dako Inc. Carpinteria, CA) as secondary antibody for 30 minutes. Sections were then incubated in avidin-biotin complex at 1 : 25 dilution (Vector Labs, Burlingame, CA) and developed using diaminobenzidine (DAB) as chromogen. After washing, the sections were counter-stained with hematoxylin and coverslipped. Negative controls were carried out by incubation in the absence of the primary antibody. Podocytes were identified by positive DAB staining under light microscopy.

2.4. Statistical Analysis. Difference in clinical variables between more than two groups was determined by Kruskal-Wallis method, differences between two groups were determined by Mann Whitney method. Analyses were performed with STATA Version 8.2 and GraphPad Prism Version 5.02 for Windows software, and results were considered statistically significant if $P < 0.05$. For test characteristics of podocyturia, sensitivity, specificity, positive predictive value, and negative predictive value were calculated for each high risk diagnosis.

3. Results

In total, 56 patients were recruited. The diagnoses at time of urine collection were as follows: preeclampsia ($n = 28$), eclampsia ($n = 1$), chronic hypertension ($n = 3$), gestational hypertension ($n = 6$), Type I DM ($n = 1$), Type II DM ($n = 1$), gestational DM ($n = 4$), connective tissue disorder ($n = 1$), marginal previa ($n = 1$), chromosomal anomaly ($n = 1$), and uncomplicated pregnancy ($n = 9$). The clinical characteristics of all subjects are described in Table 1. Podocyturia (Figures 1(a) and 1(b)) was present in 11 out of 29 (38%) patients with preeclampsia/eclampsia, 3 out of 9 (33%) with chronic and gestational HTN, and 3 out of 6 (50%) with gestational DM and Type I/II DM (Table 2). Among patients categorized as "other", 2 (marginal previa and chromosomal anomaly) out of 3 patients exhibited podocyturia (66%). In contrast, 0 out of 9 patients (0%) with uncomplicated pregnancies demonstrated podocyturia. Based on these findings, we calculated the sensitivity and specificity of podocyturia for preeclampsia to be 38% and 70%, as compared to women of HTN of any type, in whom it was 33% and 66%, respectively (Table 3). The sensitivity and specificity for DM of any type were 50% and 68%, respectively. The positive predictive value was 57% for preeclampsia, as compared with 15% for both HTN of any type and DM of any type. The negative predictive value, however, was poor for preeclampsia at 51%, as compared with 83% and 91% for HTN of any type and DM of any type, respectively.

4. Discussion

Podocyturia is well described in many glomerular diseases such as Type I DM, IgA nephropathy, lupus nephritis, and membranous nephropathy [5, 6]. Though endothelial injury is thought to be the main lesion in preeclampsia, more recently, derangements of the podocyte with downregulation of selected podocyte-specific proteins in renal biopsies and

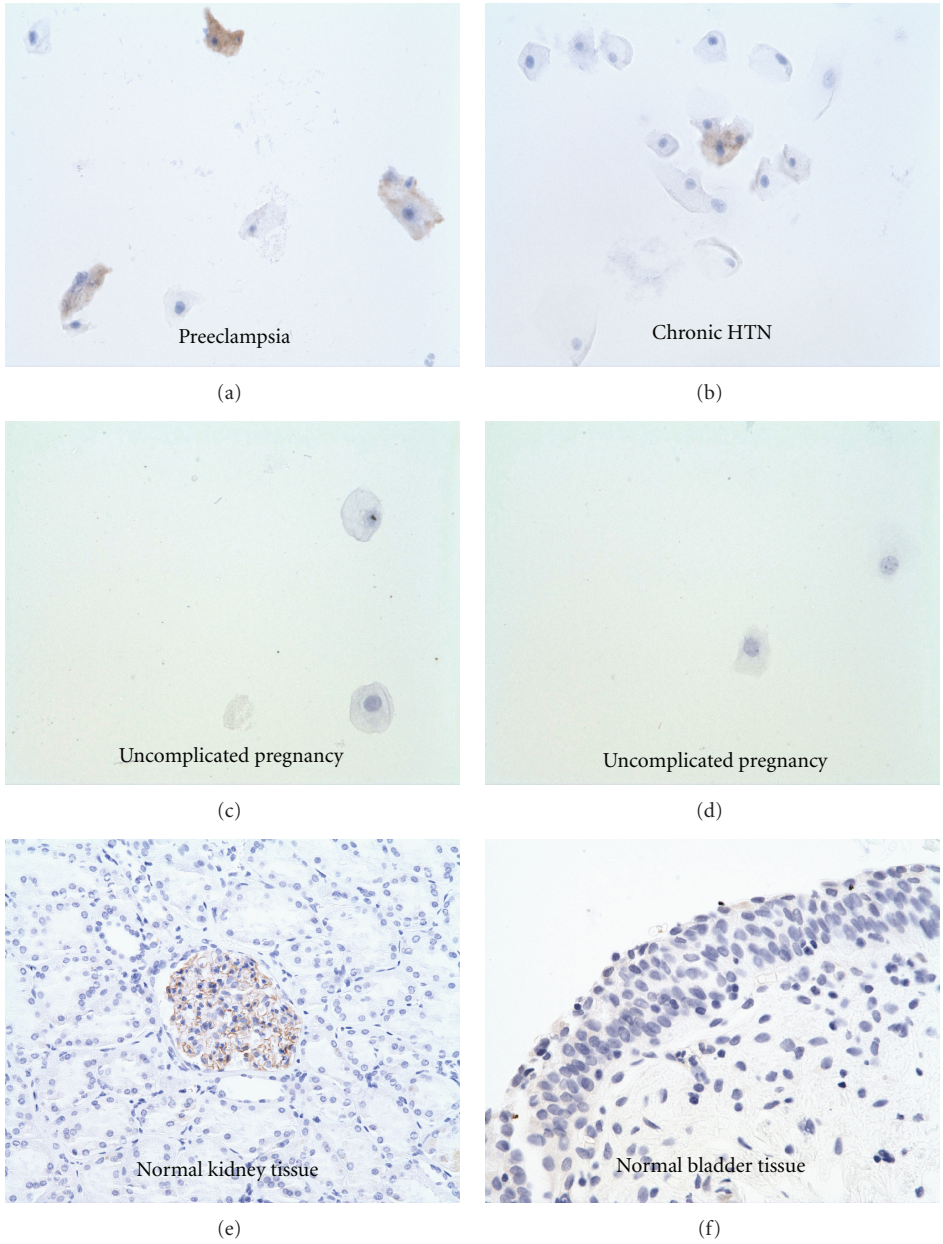

Figure 1: Podocyturia in high risk and uncomplicated pregnancies. (a) and (b) representative images of podocyturia identified by positive synaptopodin staining, (c) and (d) Representative images of negative staining in uncomplicated pregnancies, (e) synaptopodin staining in normal kidney tissue as positive control, and (f) synaptopodin staining in normal bladder tissue as negative control.

presence of nephrin and podocalyxin (podocyte specific proteins) in the urine have been described [2, 7]. In this study, we sought to describe the presence of podocyturia in preeclampsia and other high risk pregnancy states using the podocyte marker synaptopodin for identification. We found that podocyturia was neither sensitive nor specific in making this diagnosis. These results are in contrast to an earlier study by Garovic et al. [2]. Though the exact reason for this discrepancy is unclear, immunofluorescent staining of podocyte-specific proteins, in general, does not appear to be an accurate tool to identify podocytes, as these podocytes may be parietal in origin [8] and may also be apoptotic [9]. Thus, the utility of urinary podocytes to detect ongoing glomerular damage in women with preeclampsia as previously suggested [10] is unclear. Furthermore, our study showed presence of podocyturia in other high-risk pregnancy states such as DM (gestational or Type I/II), HTN (gestational or chronic). This finding is not unexpected since podocyturia and urinary podocyte mRNA have been described in nonpregnant diabetic patients [6] and in nonpregnant hypertensive patients [11] respectively. Interestingly, the number of urinary podocytes has also shown a statistically significant correlation with blood pressure but not proteinuria in preeclampsia [12].

Table 1: Patient Characteristics.

Variable	Normal (9)	Preeclampsia/eclampsia (29)	[a]HTN-chronic/gestation (9)	[b]DM-Type I/II/Gestational (6)	[c]Other (3)	*P* value
Maternal age (yr)	29.8 ± 4.4	27.9 ± 4.5	29.7 ± 4.3	28.5 ± 3.5	30.7 ± 6.9	0.52
Gestational age (wk)	37.4 ± 1.5	32.1 ± 2.7	32.3 ± 2.5	27.8 ± 6.9	26.6 ± 1.5	<0.0001
Systolic blood pressure (mmHg)	114.5 ± 12.0	156.6 ± 17.8	158.5 ± 27.0	120.8 ± 6.3	126.5 ± 6.6	<0.0001
Diastolic blood pressure (mmHg)	72.4 ± 7.2	95.0 ± 8.25	92.6 ± 8.0	75.1 ± 3.7	68 ± 9.8	<0.0001
[d]Proteinuria (mg/24 hr)	149 ± 0	1099 ± 973	150 ± 3.5	118 ± 50	109 ± 68	<0.0001

[a]HTN: hypertension.
[b]DM: diabetes mellitus.
[c]Other: diagnoses marginal previa (1), chromosomal anomaly (1), connective tissue disorder (1).
[d]24-hour proteinuria extrapolated from a urine dipstick value as described in methods section.

Table 2: Presence of podocyturia in various pregnancy categories.

	Podocyte positive	Podocyte negative	% Positive
Preeclampsia/eclampsia (29)	11	18	38%
HTN-Gestational/chronic (9)	3	6	33%
DM: any type (6)	3	3	50%
[a]Others (3)	2	1	66%
[b]Controls (9)	0	9	0%

[a]Other: diagnoses of marginal previa (1), chromosomal anomaly (1), connective tissue disorder (1).
[b]Controls: uncomplicated pregnancies.

Table 3: Test characteristics for podocyturia.

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Preeclampsia/eclampsia	38%	70%	57%	51%
HTN (any type)	33%	66%	15%	83%
DM (any type)	50%	68%	15%	91%

Though podocyturia might help shed light on the pathophysiology of preeclampsia, we feel that its clinical utility is limited. There are several limitations in relying on the podocytes as biomarkers. Identifying podocytes in the urine is highly laborious and is technically challenging. Both cytospin methods (as was performed in this study) and cultivation of podocytes in culture are fraught with difficulties and are not cost-effective. It may not be easy to eliminate podocyte cell debris when counting podocytes from cytospin specimen of fresh sediment [9]. Growing urinary podocytes in cell culture, on the other hand, are frequently limited by bacterial or fungal contamination as the urine may not have been collected under sterile conditions. Furthermore, these cells may proliferate, undergo apoptosis, or not attach to the culture dish, thereby falsely representing the true podocyte count [9]. Interobserver bias poses yet another obstacle as it requires a highly trained cytologist to correctly identify these cells.

Since clinical guidelines are available to diagnose preeclampsia, some have questioned whether the addition of a urinary marker is necessary. We feel that the utility of this marker becomes important when the diagnosis of preeclampsia is in question and when the clinical scenario is complicated by the presence of preexisting HTN, DM, or other glomerular diseases such as lupus nephritis. In those cases, the treatment would gear towards the underlying condition, in addition to supportive care. Thus, a specific marker that is discovered through our understanding of the pathophysiology of preeclampsia remains crucial. Whether podocyturia is that marker remains unanswered.

5. Limitations of the Study

The major limitation of our study is the small sample size. Our goal was to confirm the previously shown high sensitivity and specificity of podocyturia in preeclampsia. Though a larger sample size would be ideal, we feel that of our total sample size of 56 with 29 patients diagnosed with preeclampsia/eclampsia, and 18 patients with other high-risk diagnoses, is an adequate sample size to demonstrate that podocyturia lacks sensitivity and specificity to be a diagnostic marker of preeclampsia.

6. Conclusions

We discovered that podocyte loss is present not only in preeclampsia but in other high risk pregnancy states. In addition, podocyturia was not found in a majority of patients diagnosed with preeclampsia. We realize our finding of a relatively low sensitivity and specificity is not conclusive. However, our findings raise an important note of caution of relying on the limited findings in the literature regarding the predictive value of podocyturia in preeclampsia and encourage larger studies. Given our current knowledge of the complex pathophysiology of the disease such as the significant role of vascular endothelial growth factor (VEGF) signaling in maintaining a healthy endothelium and cross talk between the podocyte and vascular endothelial cell [13], it is unlikely that any single test or cell type will be able to predict preeclampsia. A panel of biomarkers reflective of this complexity may be ideal for diagnosis.

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Review Article

Preeclampsia, Hypoxia, Thrombosis, and Inflammation

Amir A. Shamshirsaz, Michael Paidas, and Graciela Krikun

Department of Obstetrics, Gynecology and Reproductive Sciences, School of Medicine, Yale University, New Haven, CT 06520, USA

Correspondence should be addressed to Graciela Krikun, graciela.krikun@yale.edu

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Reductions in uteroplacental flow initiate a cascade of molecular effects leading to hypoxia, thrombosis, inflammation, and endothelial cell dysfunction resulting in untoward pregnancy outcomes. In this review, we detail these effects and their relationship to preeclampsia (PE) and intrauterine growth restriction (IUGR).

1. Introduction

PE is universally defined as hypertension and significant proteinuria developed at or after 20 weeks of pregnancy in an otherwise normotensive woman [1–3]. PE is a multisystem disorder which complicates 3–14% of all pregnancies and about 5–8% of pregnancies in the United States [1]. The disease is mild in 75% of cases in the United States, and severe in 25% of cases [4]. Ten percent of PE occurs in pregnancies less than 34 weeks of gestation. The incidence of PE has risen in the USA in the last decades [5]. This finding might be related to an increased prevalence of predisposing disorders, such as maternal age, chronic hypertension, diabetes, prepregnancy obesity, and multiple births [5–7]. Overall, 10%–15% of direct maternal deaths are associated with PE in low- and middle-income countries and the proportion is similar in high-income countries [8, 9]. Furthermore, severe PE is a major cause of maternal morbidity (i.e., stroke and liver rupture) and negative long-term outcomes (i.e., cardiovascular disease and diabetes mel litus) as well as adverse perinatal effects, such as prematurity and intrauterine growth restriction [5, 10].

While much research has been devoted toward this topic, the cause of PE still remains elusive. Two different theories have emerged: (1) vascular-ischemic origin of PE and (2) impaired immune response [11]. A current hypothesis unifies these concepts where an altered immune response leads to disturbed placental function early in pregnancy with consequent syncytiotrophoblast ischemia and shedding of products that extensively damage endothelial integrity. This in turn results in an exponential production of multiple factors such as cytokines and growth factors leading to the clinical manifestations of PE [11]. How the immune response can activate the cascade process is still unknown but it is proposed to act in synergy with additional exacerbating factors such as predisposing maternal and ambient factors [12].

2. Angiogenic Factors and PE

Angiogenic factors and their receptors are important regulators of placental vascular development [13]. The most widely studied serum markers for PE, to date, are vascular endothelial growth factor (VEGF) and placental growth factor (PlGF). Antagonists include soluble fms-like tyrosine kinase 1 (sFlt-1, also known as sVEGFR1), and soluble endoglin (sEng) [13].

sFlt-1 is a truncated splice variant of the membrane-bound Flt1; it consists of the extracellular binding domain without the intracellular signaling domain. Several studies demonstrated the association of increased sFlt-1 with PE [14, 15].

Evidence for the involvement of sFlt1 in the occurrence of PE was initially provided by an animal model in which gravid rats were infected with a recombinant adenovirus encoding sFlt1 and compared to animals infected with the empty control adenovirus. The animals infected with sFlt1 developed

a syndrome highly reminiscent of human PE: hypertension and proteinuria due to a glomerular endotheliosis [14, 16].

Similarly, sEng is a truncated form of receptor for two subtypes of transforming growth factor beta (TGFβ) specifically, TGFβ1 and TGFβ2 which are highly expressed by vascular endothelial cells and syncytiotrophoblasts. Like sFlt1, soluble endoglin (sEng) is an antiangiogenic factor capable of inhibiting capillary tube formation *in vitro* [17]. Soluble Eng also increases vascular permeability; overexpression of both sFlt1 and sEng in rodents results in capillary permeability in the lungs, kidneys, and liver [18]. Overexpression of both sEng and sFlt1 in pregnant rats develops nephrotic-range proteinuria, severe hypertension, biochemical evidence of HELLP ("H" for hemolysis, "EL" for elevated liver enzymes, and "LP" for low platelet count), and intrauterine growth restriction of the pups [19, 20].

PlGF concentration during pregnancy increases during the first 30 weeks of gestation, and then decreases [13]. Longitudinal studies have shown that a relatively low PlGF concentration (which could be explained by a high sFlt1 concentration) is also a characteristic feature of PE. Decreased levels of urinary PlGF and PIGF : sFlt-1 ratio during midgestation have been proposed as a predictive model for development of clinical PE, and quantification of sFlt-1 levels has correlated directly with severity of disease and inversely with time to onset of proteinuria and hypertension [21].

The exact molecular basis for placental dysregulation of these factors remains unknown but hypoxia is likely an important regulator [22]. Other factors such as alterations in the renin-angiotensin-aldosterone axis, immune maladaption, excessive shedding of trophoblast debris, oxidative stress, and genetic factors likely contribute to the pathogenesis of the abnormal placentation [22]. To date the most successful treatment for PE is delivery.

3. Preeclapmpsia and Cathecol-O-methyltrasferase

Cathecol-O-methyltrasferase (COMT) catalyzes the O-methylation of various circulating hormones such as catecholamines and catecholestrogens [23, 24]. In the placenta, COMT metabolizes certain forms of circulating estradiols to the molecule 2-methyl estradiol (2-ME). This estradiol metabolite has several effects, one being the destabilization of hypoxia-inducible factor- (HIF-) 1α3 in the cytoplasm. HIFs are heterodimeric proteins that mediate the effects of hypoxia on gene expression by upregulating transcription of target genes including sFlt1 [25]. This role of COMT in maintaining oxygen balance suggests that COMT might somehow be involved in the pathogenesis of PE.

To better elucidate the role of COMT in PE, a genetic COMT knockout (COMT−/−) mouse model was recently developed. Interestingly, pregnant COMT−/− mice developed a PE-like phenotype characterized by proteinuria, increased blood pressure, and histopathological changes in the placenta and kidney. This phenotype was accompanied by lower plasma concentrations of 2-ME and higher placental protein levels of HIF-1a [26]. Restoration of 2-ME in COMT−/− mice decreased HIF-1a and ameliorated the preeclamptic phenotype [26]. Together, these results suggest that COMT and 2-ME deficiency might play a significant role in the development of PE.

The exact mechanism by which 2-ME prevents PE remains unknown. Although 2-ME was found to suppress HIF-1a and sFlt1, recent experiments suggest that there might be several other mechanisms through which 2-ME promotes vascular health [27].

Lastly, recent evidence suggests that 2-ME is necessary for cytotrophoblast invasion of the maternal decidua and therefore contributes to the prevention of PE by promoting normal placental vascular formation [28]. Further studies on this topic will be required to ascertain a more exact mechanism of action.

4. PE and Complement Factors

The complement system, composed of over 30 proteins that act in concert to protect the host against invading organisms, initiates inflammation and tissue injury [29] and normally has a protective role toward off-infection. Complement activation promotes chemotaxis of inflammatory cells and generates proteolytic fragments that enhance phagocytosis by neutrophils and monocytes. There is scant information about complement activation in normal and abnormal human pregnancy [30]. During normal gestation, serum levels of C3, C4, and total hemolytic complement (CH50) gradually increase 10%–50% [31]. Studies have shown significant elevations in levels of Bb, C3a, C4d, and soluble C5b-9 in preeclampsia, indicating excess activation of both the classical and alternative complement pathway [32]. Lynch et al. conducted a large prospective study in human pregnancy to investigate whether elevated levels of complement activation fragment Bb (reflecting alternative complement pathway activation) at a single point in early pregnancy (less than 20 weeks gestation) were predictive of preeclampsia later in pregnancy. Adjusted for other risk factors, women with higher levels of Bb in early pregnancy were almost four times more likely to develop preeclampsia later in pregnancy compared with women with levels less than the top decile in early pregnancy [33]. Products of complement pathway have been found in deciduas, chorionic villi, and as subendothelial deposits in vessel walls in normal and preeclampsia [34, 35]. A recent study reported that the presence in C5b-9 MAC on trophoblasts was associated with fibrin deposits at sites of villous injury *in vivo* in normal placentas, but especially in placentas from pregnancies complicated by IUGR or preeclampsia [36]. It has been demonstrated that in normal pregnancies, complement regulatory proteins that are highly expressed on trophoblast membranes prevent excessive complement activation (membrane cofactor protein [MCP (CD46)], decay accelerating factor [DAF (CD55)], and CD59)) as well as circulating complement regulatory proteins (complement factor H [CFH], C4b binding protein, and complement factor I [CFI]) [37, 38]. Defective regulation of the complement system allows for the excessive complement activation that leads to placental damage,

abnormal placental development, generalized endothelial activation, and the release of antiangiogenic factors toxic to the fenestrated endothelium of glomeruli, the choroid plexus, and liver sinusoids—a sequence of events that culminates in clinical preeclampsia [39]. The link between complement activation and pathogenic events in preeclampsia identifies potential biomarkers to predict patients at risk for preeclampsia and new targets to prevent its complications.

5. PE and Coagulation Factors

As implied in the preceding section, insufficient and/or inadequate trophoblastic invasion of the maternal spiral arteries leads to reduced uteroplacental blood flow causing focal decidual hypoxia, and thus VEGF [40–42]. This in turn results in activation of the decidual endothelial cells and the aberrant expression of tissue factor (TF) [40, 43]. In addition, under pathological conditions, other cells, such as macrophages present in the maternal fetal interface, can also generate TF. TF generates thrombin that further induces endothelial cell TF expression and inflammatory cytokines [40]. Both VEGF and TF induce aberrant angiogenesis and poor vessel maintenance reflected by endothelial cell fenestrations and induction of a prothrombotic surface and uteroplacental vascular insufficiency [44].

Under physiological conditions, TF is not expressed by endothelial cells. By contrast, endothelial TF expression is observed in pathologic conditions such as sepsis, atherosclerosis, and rapid but malformed vessel growth associated with malignancies and allograft rejection [45–47]. Indeed, expression of TF by the endothelium is a pathological consequence of cross-talk between coagulation and inflammatory cytokines [45]. Expression of TF transforms endothelial cell membrane from an anticoagulant to a procoagulant surface and promotes intravascular thrombosis [48, 49]. Interestingly, the uteroplacental vasculature of pregnancies complicated by PE and IUGR display vascular features similar to those seen in allograft rejection [50]. A recent study by Di Paolo et al. demonstrated high expression of TF mRNA in the vascular endothelium of vessels in the decidua basalis of pregnancies complicated by PE and IUGR but not in normal pregnancies [51].

Tissue factor, the critical initiator of the coagulation cascade, is induced during pregnancy in the maternal decidua [52, 53] and is plentiful in the placenta and amniotic fluid [54–57]. It has been well documented that patients with diseases leading to aberrantly increased circulating TF expression have a much higher rate of vascular thromboembolism (VTE) [58–61]. Specifically, patients are at risk for cardiovascular diseases, sepsis, hematologic, and coagulation disorders such as disseminated intravascular coagulation [62–67]. In these diseases, as well as in cancer and diabetes, plasma TF is related to increased blood thrombogenicity [67]. Interestingly, plasma levels of thrombomodulin activity, tissue factor activity, and procoagulant phospholipids were significantly elevated in women with PE versus normal pregnant and nonpregnant women [68]. Moreover, the circulating TF has been identified on circulating microparticles (MPs). These MPs are small vesicles released from injured or activated cells, primarily leukocytes and endothelial cells [58, 69]. TF-bearing MPs in pregnancy also arise from syncytiotrophoblasts [70] and excess syncytiotrophoblast microparticles have been found in the circulation of women with PE [71]. Unfortunately, VTE is one of the leading causes of morbidity and mortality during pregnancy [72]. A higher prevalence of risk factors for VTE has been found in women with PE and fetal loss [73].The principal factors that result in venous thrombosis consist of the classic triad of Virchow: venous stasis, vascular damage, and hypercoagulability, all of which occur during normal pregnancies [74]. A shift of the hemostatic balance in the direction of hypercoagulability during pregnancy is believed to be evolutionarily advantageous in reducing hemorrhage, however, this could also contribute to the increased risk of venous thromboembolic processes [75]. Studies from our laboratory have demonstrated that TF is normally expressed by decidual cells [76]. Indeed, immunohistochemical staining for TF has been observed in the decidualized endometrial stromal cells, but not in endothelial cells [77–79]. In contrast, we noted that basal plate uteroplacental vessel segments from cases of low birth weight with villous evidence of maternal uteroplacental malperfusion had increased percent of strong positive endothelial immunostaining for TF [40].

6. Thrombin Activation of Endometrial Endothelial Cells

Vascular injury initiates clotting when plasma-derived factor VII binds to the extracellular domain of perivascular cell membrane-bound TF. The resulting TF/VIIa complex promotes hemostasis via a series of changes initiated by the extracellular cleavage of prothrombin to thrombin [76]. The aberrant expression of TF in vessels from deciduas with activated endothelium led us to study this phenomenon in further detail. We proposed that aberrantly expressed TF in decidual vessels would in turn result in thrombin production and further endothelial activation. *In vitro* experiments were carried out with cultured human endometrial endothelial cells (HEECs) [40]. Treatment with 2.5 U of thrombin demonstrated the induction of TF protein and mRNA expression [40]. Moreover, thrombin-induced inflammatory cytokine expression in HEECs. Specifically thrombin significantly upregulated mRNA expression for regulated upon activation, normal T-cell expressed, and secreted (RANTES) chemokine, growth-related oncogene (Gro)-β, Gro-γ, granulocyte chemotactic protein (GCP)-2, granulocyte-macrophage colony stimulating factor (GM-CSF), monocyte chemotactic protein-1 (MCP-1), interleukin-8 (IL-8), and macrophage inflammatory protein 3α (MIP3α) [40]. This increase in proinflammatory chemokines are expected to result in endothelial dysfunction or inappropriate endothelial cell activation which are the most common clinical manifestations in PE, including enhanced endothelial-cell permeability and platelet aggregation [80]. Moreover, the overexpression of these chemokines is consistent with observations made in our laboratory [81, 82] as

well as others [83, 84] indicating that the preeclamptic decidua contains an excess of macrophages [82].

7. Summary

To date, no accurate test exists for predicting PE. In recent years, it has become accepted that early-onset and late-onset PEs are associated with different biochemical, histological, and clinical features [85]. Moreover, markers such as sFLT, sEng, products of fetal and placental origin, markers of renal or endothelial damage, or markers of oxidative stress are secondary to the pathophysiological changes that precede the clinical onset of PE [85]. Nonetheless, a combination of markers may increase the detection accuracy earlier in the pregnancy and hopefully allow for more effective prophylactic strategies.

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Review Article

Prevention of Vascular Dysfunction after Preeclampsia: A Potential Long-Term Outcome Measure and an Emerging Goal for Treatment

Merzaka Lazdam,[1] Esther F. Davis,[1] Adam J. Lewandowski,[1] Stephanie A. Worton,[2] Yvonne Kenworthy,[1] Brenda Kelly,[2] and Paul Leeson[1]

[1] *Department of Cardiovascular Medicine, Oxford Cardiovascular Clinical Research Facility, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK*
[2] *Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK*

Correspondence should be addressed to Paul Leeson, paul.leeson@cardiov.ox.ac.uk

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Preeclampsia is increasingly being recognised as more than an isolated disease of pregnancy. In particular, preeclampsia has emerged as an independent risk factor for maternal cardiovascular disease and has recently been recognised as a risk factor for cardiovascular disease in children exposed in utero. Preeclampsia and cardiovascular disease may share important pathophysiological and molecular mechanisms and further investigation into these is likely to offer insight into the origins of both conditions. This paper considers the links between cardiovascular disease and preeclampsia and the implication of these findings for refinement of the management of patients whose care is complicated by preeclampsia.

1. Introduction

Although traditionally preeclampsia has been viewed as a condition that resolves completely with the delivery of the placenta, there is now increasing evidence that preeclampsia may constitute a condition with significant long-term health implications for both the mother and child. In particular preeclampsia has recently emerged as an independent risk factor for maternal cardiovascular disease 10–15 years after the index pregnancy [1–3]. A history of preeclampsia is therefore now considered a relevant factor in the cardiovascular risk assessment in women [4] and is associated with an increase in risk similar in magnitude to a history of dyslipidemia [5]. Meta-analysis has demonstrated that in the 10–15 years following a preeclamptic pregnancy women have an increased risk of developing hypertension (RR 3.7 95% C.I. (2.7–5.05, $P < 0.001$)), coronary artery disease (RR 2.16 95% C.I. (1.86–2.52, $P = 0.001$)), and stroke (RR 1.81 95% C.I. (1.45–2.27, $P = 0.00$) [1]. Additionally, it has been demonstrated that there is a graded relationship between the risk of cardiac disease and the severity of preeclampsia with maternal risk being greatest with early onset or severe preeclampsia [6]. Children born to preeclamptic women have also been demonstrated to have elevated blood pressure in childhood and adolescence [7–12].

This highlights potentially important pathophysiological or molecular links between preeclampsia and cardiovascular disease. Greater insight into these links may identify new opportunities to understand disease predisposition and treatment and may also raise the possibility that prevention of vascular dysfunction should be an important long-term goal of preeclampsia management. This paper will consider the current literature considering the links between cardiovascular disease and preeclampsia and the implication of these findings for refining the management of patients whose care is complicated by preeclampsia.

2. What Links Preeclampsia and Cardiovascular Disease?

The aetiology of preeclampsia remains incompletely understood, though disturbed placentation and placental functioning in early pregnancy remains the leading hypothesis [31]. During normal placental development, fetal cytotrophoblasts invade the maternal spiral arteries transforming them to high-caliber capacitance vessels providing low resistance placental perfusion adequate to sustain fetal growth [32]. However, inadequate spiral artery remodeling in preeclampsia is thought to lead to chronic placental ischaemia or intermittent flow through the narrow muscular arteries thereby creating an ischaemia-reperfusion phenomenon [33].

Reactive oxygen species and cytokines released from the ischaemic placenta trigger a systemic oxidative stress [34] and contribute to the exaggerated systemic inflammatory reaction in preeclampsia [33, 35]. Syncytiotrophoblasts undergoing apoptosis also shed increased numbers of microparticles in the maternal circulation which contribute to this process. The release of cytokines and acute phase proteins, such as TNFα, leptin, and PAI-1, not only enhance the inflammatory response but also induce some of the observed metabolic disturbances in preeclampsia including insulin resistance, lipolysis, and hyperlipidaemia [33, 34].

Furthermore, hypoxic oxidative stress and inflammatory stimuli provoke the release of antiangiogenic factors (via NF-κB pathways) [33, 36–38]. Soluble Fms-Like Tyrosine kinase-1 (sFLT-1), a circulating truncated form of VEGF receptor, binds and reduces levels of VEGF and PlGF in the maternal circulation thereby inhibiting angiogenesis and vasodilatation [39]. This aberrant vasculature is thought to lead to a cascade of events which end in symptomatic systemic endothelial dysfunction [40]. Soluble endoglin, a TGF-β coreceptor, is one of the antiangiogenic factors that enhances vascular permeability and may possibly affect nitric oxide synthesis and vasodilatation via altered downstream signalling in the TGF-β pathway contributing to the endothelial dysfunction [35] (Figure 1).

Consistent with these biological changes, reduced endothelial-dependent vasodilatation in conduit and resistance arteries [21, 41–47] has been demonstrated in women who have preeclampsia. They also appear to have increased arterial stiffness [13], increased atherosclerosis [48], and diminished capillary density [49]. Biochemical markers of endothelial activation and dysfunction are also elevated in preeclampsia [34, 50]. Maternal endothelial dysfunction is also present in conduit vessels before the onset of clinical disease [42] and up to three years after an affected pregnancy [24, 51, 52]. The increase in cardiovascular risk following preeclampsia may be a consequence of the pro-atherogenic impact of persistent endothelial dysfunction, a result of subclinical endothelial injury at the time of pregnancy, or to preexisting differences in endothelial function that predispose to both conditions (Figure 2).

Dysfunction of the vascular endothelium is a key factor in the development of atherosclerotic cardiovascular disease and has been demonstrated to precede clinically identifiable structural changes in the vasculature [53]. Peripheral dysfunction of the vascular endothelium has been demonstrated to correlate with increased risk of clinical events [54] and all cause mortality [55] and associates with many traditional cardiovascular risk factors including hypertension, diabetes mellitus, insulin resistance, hypercholesterolemia, and smoking [56]. In addition to this, endothelial dysfunction has been demonstrated in young adults predisposed to hypertension without clinical evidence of arterial disease [57]. Endothelial health and nitric oxide synthase activity are crucial in modulating arterial distensibility [58–60] and carotid intima media thickness [61, 62] independent of risk factors [62] as well as myocardial hypertrophic responses in animals [63–65]. Left ventricular mass, another powerful independent predictor of mortality and morbidity in adults free of clinical disease [66], has a graded relationship with vascular endothelial vasomotor responses in hypertensive adults [67–72]. The strong relationship between endothelial dysfunction and cardiovascular outcomes and risk factors make it an interesting pathophysiological endpoint in the evaluation of therapies designed to modify long-term cardiovascular risk.

3. Maternal Vascular Function as an Intermediate Endpoint in the Management of Preeclampsia

As women who have experienced preeclampsia constitute a relatively young group, and there is potentially a prolonged period between exposure and clinical outcome, intermediate measures which may indicate a potentially modifiable change in risk are of particular value. Noninvasive measures of vascular function are widely used as surrogate markers of cardiovascular risk [73, 74]. For example, increasing arterial stiffness, demonstrated by an increase in pulse wave velocity, is an antecedent factor in elevated blood pressure, predicts the future cardiovascular risk of adults, and correlates strongly with the presence of atherosclerosis [75]. Similarly, early evidence of atherosclerosis demonstrated by increased thickness of the arterial wall is correlated with coronary artery disease and is predictive of future infarction and stroke [75, 76]. Changes in these parameters may therefore also offer unique insight into the cardiovascular risk of women and children following a preeclamptic pregnancy.

Several relatively small-scale studies have also demonstrated vascular dysfunction after pregnancy complicated by preeclampsia with evidence of endothelial dysfunction in the macrocirculation [14, 16, 18, 22–24, 26] up to a median of 3 years and the microcirculation up to 25 years later [15, 27, 30], as well as increased arterial stiffness [13, 14, 16, 17] up to almost 5 years after the index pregnancy and atherosclerosis over 3 months postpartum [19]. Elevation of systemic biomarkers of endothelial injury and inflammation have also been described between 6 weeks [77] and 20 years [78–80] following preeclampsia. The characteristics and results of current literature considering the impact of preeclampsia on vascular structure and function are summarised in Table 1. There is, however, some disparity in results with some studies demonstrating no change in vascular function following

Table 1: Studies assessing the long-term impact of preeclampsia on vascular function. AIx (augmentation index) and PWV (pulse wave velocity) are robust surrogate markers of aortic stiffness. Endothelial-mediated vasodilatation was measured in conduit arteries by FMD (flow-mediated dilatation) and in the resistance arteries using VOP (venous occlusion plethysmography) to measure FBF (forearm blood flow) or peripheral arterial tonometry (PAT) to measure RHI (reactive hyperaemic index). Mircovascular endothelial responses were quantified in the microcirculation using LDF (laser Doppler flowmetry) and iontophoresis. SGA refers to small for gestational age.

Study	Subjects (Preeclampsia/control)	Interval after delivery	Vascular measures	Results in women with previous preeclampsia
Aortic stiffness				
Robb et al. 2009 [13]	15/22	7 weeks	AIx, PWV	Elevated PWV and AIx.
Yinon et al. 2010 [14]	24/16	6–24 months	AIx	Increased AIx in women with early onset preeclampsia.
Evans et al. 2011 [15]	18/50	6–36 months	PWV	No significant differences in central arterial stiffness.
Páez et al. 2009 [16]	20/20	2 years	PWV, AIx	Elevated PWV and AIx.
Elvan-Taşpinar et al. 2005 [17]	44/46	4–56 months	PWV	Elevated PWV.
Lampinen et al. 2006 [18]	30/21	5-6 years	AIx	No significant differences in AIx.
Subclinical atherosclerosis				
Blaauw et al. 2006 [19]	22/22	≥3 months	Femoral and carotid IMT	Increased IMT with early onset preeclampsia.
Conduit artery endothelial function				
Kuscu et al. 2003 [20]	15/11	2 and 6 weeks	FMD	Reduced FMD both during pregnancy and postpartum (no control data postpartum).
Noori et al. 2010 [21]	45/21	12 weeks	FMD	No significant differences in FMD compared to controls.
Yinon et al. 2010 [14]	24/16	6–24 months	FMD	Reduced FMD in women with early onset preeclampsia.
Hamad et al. 2007 [22]	18/17	15 ± 3 months	FMD	Reduced FMD and endothelial independent dilatation in women with severe preeclampsia.
Germain et al. 2007 [23]	25/22	16 ± 3.5 months	FMD	Reduced FMD.
Páez et al. 2009 [16]	20/20	2 years	FMD	Reduced FMD.
Chambers et al. 2001 [24]	113/48	3 years median	FMD	Reduced FMD particularly in women with recurrent preeclampsia and recovery of endothelial function with ascorbic acid.
Resistance artery endothelial function				
Lommerse et al. 2007 [25]	32/10	0.64–1.6 years	VOP	No significant difference in FBF.
Agatisa et al. 2004 [26]	16/14	9.9 ± 0.5 months	VOP	Reduced endothelium-mediated FBF.
Evans et al. 2011 [15]	18/50	6–36 months	VOP	Reduced FBF in response to mental stress.
Lampinen et al. 2006 [18]	30/21	5-6 yrs	VOP	Significantly reduced FBF to acetylcholine (ACh) and sodium nitroprusside (SNP).
Kvehaugen et al. 2011 [27]	26/17	5–8 years	PAT	RHI comparable between preeclamptic women and controls. Women with SGA baby had significantly lower RHI.
Cutaneous microvascular function				
Khan et al. 2005 [28]	15/54	6 weeks	LDF and iontophoresis	No significant differences between women in endothelial dependent or independent microvascular dilatation.
Blaauw et al. 2005 [29]	25/23	7.0 ± 2.8 months	LDF and iontophoresis	Greater microvascular vasodilator responses in preeclampsia.
Ramsay et al. 2003 [30]	10/10	16–25 years	LDF and iontophoresis	Reduced response to endothelial-dependent and -independent dilatation.

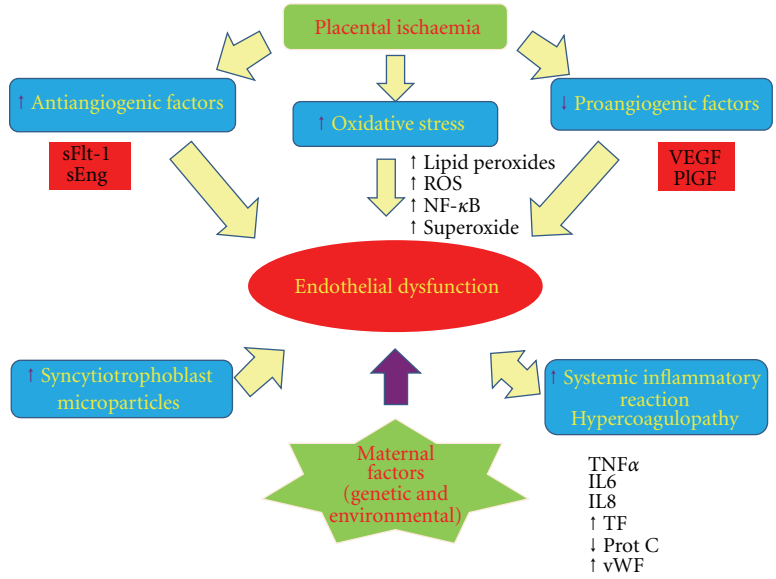

Figure 1: Molecular and vascular mechanisms of endothelial dysfunction in preeclampsia. Defective placentation, a common feature of preeclampsia, triggers a cascade of events including oxidative stress and exaggerated inflammatory reaction and angiogenic imbalance which exacerbate endothelial dysfunction. Impaired endothelial function plays a central role in the clinical manifestations of preeclampsia such as hypertension and proteinuria.

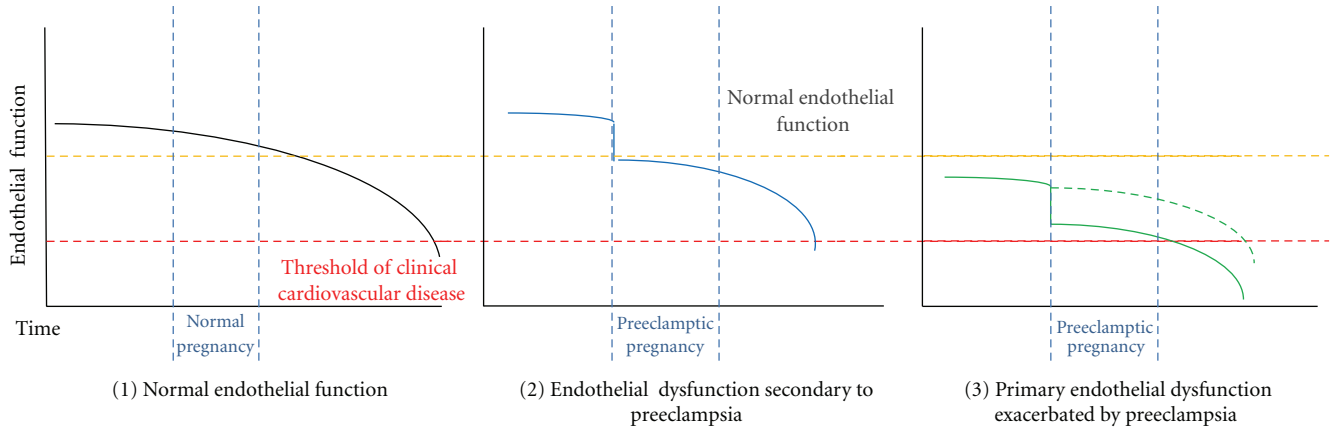

Figure 2: Theoretical timelines of impairment of endothelial function and development of cardiovascular disease following preeclamptic pregnancy. (1) In the normal individuals there is a gradual age-related reduction in endothelial function, which can be exacerbated by the presence of cardiovascular risk factors and associates with the future risk of clinical cardiovascular disease. (2) Women who experience preeclamptic pregnancies are known to have impaired endothelial function during pregnancy and up to 3 years following an affected pregnancy. It is possible that these women begin life with normal endothelial function, which is acutely impaired during a preeclamptic pregnancy. This followed by ongoing age-related decreases in endothelial function may relate to the increased incidence of cardiovascular disease in these individuals. (3) Alternatively, women who develop preeclampsia may have primary endothelial dysfunction which both puts them at risk of preeclampsia, this may then be exacerbated by the preeclamptic pregnancy (solid line), or simply persist (dotted line), in either case leading to higher incidence of cardiovascular disease.

preeclampsia [21, 25, 28, 81]. This may in part reflect heterogeneity in patient cohorts, severity of preeclampsia, size of cohorts, the timing of the studies following the affected pregnancy, as well as differences in the vascular beds studied and the techniques employed. In the future more detailed long-term follow-up studies of such women may clarify changes in cardiovascular physiology and inform future treatment.

4. Offspring Vascular Function Following Preeclampsia

Even more than their mothers, children born following a preeclamptic pregnancy, constitute a cohort where early life preventative strategies may have a profound impact on future cardiovascular risk. Furthermore, adults whose mothers had preeclampsia themselves have a higher risk

of the condition. Therefore, in this group evidence of subtle changes in vascular physiology indicating changes in risk are of particular importance. The body of literature considering cardiovascular outcomes in the offspring of preeclamptic pregnancies is sparse when compared to that considering maternal health. A single 60-year-follow-up study of individuals born to preeclamptic women demonstrated an increased risk of stroke in later life (RR 1.9 95% C.I. (1.2–3.0, $P = 0.01$)) [82]. Offspring of hypertensive pregnancies have also been shown to have an increased risk of hypertension as adults [82, 83] as well as having increased, although not pathological, blood pressure in childhood and adolescence [84]. Meta-analysis suggests that the magnitude of this increase in young individuals for systolic blood pressure is 2.3 mmHg and for diastolic blood pressure is 1.7 mmHg [84]. Although such increases are unlikely to be clinically recognisable, it may have significant public health significance as a 2 mmHg rise in systolic blood pressure has been associated with a 7% increase in ischaemic heart disease mortality and a 10% increase in stroke [85].

Preterm offspring born to hypertensive pregnancies demonstrate a distinct cardiovascular phenotype, characterised by reduced conduit artery endothelial function and increased evidence of early atherosclerosis, compared to individuals born preterm to normotensive women [7]. A finding which has now been replicated in other groups [27, 86, 87] and may be similar to the vascular dysfunction seen in women following preeclamptic pregnancies [27]. Although the underlying mechanisms remain unknown, there is substantial evidence of "programming" of aspects of vascular biology during fetal development, in particular en-dothelial responses [88] and arterial stiffness [89]. The risk to the offspring is likely to be mediated through changes in maternal blood pressure, vascular resistance in the placenta, or exposure to maternal factors (such as antiangiogenic factors [90], vasoactive substances [91], and reactive oxygen species) in the fetomaternal circulation. Optimal management of preeclampsia may have indirect benefits to reduce cardiovascular risk in the offspring of such pregnancies [92]. Hence, better understanding of the long-term vascular changes in offspring of preeclampsia may allow assessment of novel and previously unrecognised long-term outcomes of preeclampsia with important public health significance.

5. Conclusions and Future Directions

It is now becoming clear that preeclampsia is more than an isolated disease of pregnancy. The long-term health implications of this condition for both the women and their children are increasingly being recognised and incorporated into clinical risk assessments [4]. Both women and children exposed to preeclampsia exhibit an adverse vascular phenotype, a propensity to subclinical atherosclerosis, and increased risk of adverse cardiac and vascular events in future life. As preeclampsia affects 2–5% of the population this altered risk is relevant to the health of 1.2 to 3 million people in the UK and 6 to 15 million people in the USA. Optimal management of preeclampsia may be able to improve short and long-term vascular outcomes in these individuals. While we remain unable to effectively prevent preeclampsia attempts to reduce its long-term impact on those exposed are of potential importance. Future studies that define the detailed cardiovascular phenotype of those exposed to preeclampsia may allow identification of potential targets for future preventative strategies. Furthermore, studies into the mechanisms underlying the altered cardiovascular phenotype may provide unique insight into pathophysiological or molecular links between preeclampsia and cardiovascular disease, which may direct us to novel treatment strategies for both conditions. Vascular dysfunction is an early marker of cardiovascular risk, correlating with future risk of cardiac events and preceding structural vascular change [53]. Improvement in vascular function would therefore be a valuable intermediate endpoint in studies aiming to reduce risk in this potentially young and generally asymptomatic population before the onset of clinical disease.

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Review Article

Pathogenesis of Preeclampsia: The Genetic Component

Francisco J. Valenzuela,[1] Alejandra Pérez-Sepúlveda,[1] María J. Torres,[1] Paula Correa,[1] Gabriela M. Repetto,[2] and Sebastián E. Illanes[1]

[1] *Departamento de Obstetricia and Ginecología y Laboratorio de Biología de la Reproducción, Universidad de Los Andes, Santiago 7620001, Chile*
[2] *Centro de Genética Humana, Facultad de Medicina, Clínica Alemana-Universidad del Desarrollo, Santiago 7620001, Chile*

Correspondence should be addressed to Sebastián E. Illanes, sillanes@uandes.cl

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Preeclampsia (PE) is one of the main causes of maternal and fetal morbidity and mortality in the world, causing nearly 40% of births delivered before 35 weeks of gestation. PE begins with inadequate trophoblast invasion early in pregnancy, which produces an increase in oxidative stress contributing to the development of systemic endothelial dysfunction in the later phases of the disease, leading to the characteristic clinical manifestation of PE. Numerous methods have been used to predict the onset of PE with different degrees of efficiency. These methods have used fetal/placental and maternal markers in different stages of pregnancy. From an epidemiological point of view, many studies have shown that PE is a disease with a strong familiar predisposition, which also varies according to geographical, socioeconomic, and racial features, and this information can be used in the prediction process. Large amounts of research have shown a genetic association with a multifactorial polygenic inheritance in the development of this disease. Many biological candidate genes and polymorphisms have been examined in their relation with PE. We will discuss the most important of them, grouped by the different pathogenic mechanisms involved in PE.

1. Introduction

Preeclampsia (PE) and its complications and associated pathologies have become one of the main causes of maternal and fetal morbidity and mortality in the world, causing nearly 40% of births delivered before 35 weeks of gestation. Moreover, PE has been strongly associated with an increased risk of later-life death due to cardiovascular disease, independent of other risk factors [1–3]. PE is present in around 5–10% of all pregnant women worldwide and despite the amount of resources invested in the research and treatment of this pathology, its development is still barely predictable and thus challenging to prevent and manage clinically.

PE constitutes a clinical spectrum that includes "maternal" PE and "placental" PE [4]. In general, placental PE involves an abnormal placentation in a healthy woman, and in maternal PE there is a normal placentation in a woman with a preexistent pathology, like cardiovascular disease, chronic arterial hypertension, or diabetes. Nevertheless, in practice, the great majority of patients who will develop PE have both types to different degrees, and rather than being two distinct types of disease, they are the extremes of the same pathologic entity. These 2 origins may explain the variability in the severity and gestational age of presentation of this syndrome. Placental PE, with a poor trophoblastic perfusion, generates oxidative stress, with liberation of trophoblast factors to maternal circulation, which will induce a secondary inflammatory response and endothelial dysfunction [5]. In maternal PE, an inflammatory response takes place, involving all the inflammatory components of circulation, including the endothelium [5].

The understanding of the underlying factors that explain the pathogenesis of PE and the early identification of the patients at risk of the disease will help in the development of preventative or early therapeutic interventions, aimed to reduce the associated morbidity and mortality during pregnancy, but also the long-term severe problems that PE may produce or is associated with.

2. Pathogenesis of PE: A Placental Originated Disease

Despite the breakthroughs in the understanding of the pathogenesis of PE, the mechanisms that finally trigger the disease are still not clearly elucidated. Nevertheless, it seems clear that the development of PE during pregnancy requires the presence of the placenta, given that this clinical syndrome will not be developed if it is not present, and it disappears soon after placental delivery [6]. In placental PE, it is also widely accepted that the physiopathological process of PE begins with inadequate trophoblast invasion early in pregnancy, which produces an increase in oxidative stress contributing to the development of systemic endothelial dysfunction in the later phases of the disease, leading to the characteristic clinical manifestation of PE, with hypertension, proteinuria, and edema.

During normal placentation, the cytotrophoblast cells form a highly invasive extravillous trophoblast (EVT) that can migrate into the decidua and invade the first third of the myometrium, inducing the remodelling of spiral arterioles to produce the low-resistance vascular system that allows a 10-fold increase in blood flow, essential for fetal growth [7–9]. The relative reduction of uteroplacental flow, secondary to abnormal placentation caused by an impaired trophoblast invasion [8], is the trigger for the development of PE [10–12]. This was first highlighted by the histopathological findings in the site of placental implantation, where 80 to 100% of patients with PE have a deficit of the physiological invasion of the maternal spiral arteries by the EVT [8, 13]. It has been postulated that the physiological changes that favor the invasive phenotype of these cells are due to the exposure of cytotrophoblast cells to a hypoxic environment. The normal concentration of oxygen in the first trimester placenta is only about 3% O_2 ($\pm$18 mm Hg) and this low oxygen milieu is believed to facilitate trophoblast invasion [14]. In the normal placentation process, the cytotrophoblast invasion is regulated by the gradient of oxygen concentration between the placenta and maternal arteries. Therefore, the hypoxic environments that face the cytotrophoblast at the beginning of the placentation change gradually to a normoxic environment as invasion takes place [15]. In pathologies with an abnormal placentation, the trophoblastic invasion is poor and limited only to spiral arteries present in superficial decidua. The mechanisms involving this poor placentation are still areas of research.

In summary, the events that lead to the development of a PE may be explained by a first stage of defective trophoblastic invasion, which occurs early in pregnancy, with uteroplacental circulation remaining in a state of high resistance during pregnancy, which can be detected by an increased resistance of the uterine arteries [16]. The persistence of a state of underperfusion produces placental hypoxia and local oxidative stress, resulting in a systemic inflammatory response and endothelial dysfunction, leading to the onset of the clinical symptoms of PE [4]. The first stage is difficult to diagnose, while the second is the clinical syndrome itself.

3. Prediction of PE

Numerous methods have been used to predict the onset of PE with different degrees of efficiency. These methods have used fetal/placental and maternal markers in different stages of pregnancy in order to predict the disease. The fetal/placental markers could be divided in (1) trophoblast invasion (PLGF, IGFBP-1, PAPP-A, Doppler ultrasound, and HLA-G), (2) placental hypoxia (sFlt-1, VEGF, PLGF), (3) reactive oxygen species (lipid peroxide), and (4) placental function (activin/inhibin, CRH/CRHBP, and PAI-2). The maternal markers that have been used could be classified as (1) metabolic syndrome (BMI, Leptin, insulin, and glucose), (2) endothelial function (PAI-1, Fibronectin, VCAM/ICAM), (3) prooxidants (8-epi-PGF2a), (4) antioxidant reserve (vitamins C and E), and (5) immune function (AT-R autoantibodies) [17].

Despite the improvements in the prediction of the disease, there is no treatment that reverses this pathology once it has begun. This is mainly explained by the fact that the different tests used to predict PE have a better performance after the first trimester of pregnancy, a period in which the intervention to decrease the prevalence of the disease has proved to be ineffective [18]. This is why more research is needed to develop clinical tools that allow a prediction in even more early stages, or even before the patient gets pregnant. Moreover, it should be noted that the PE has a clear genetic component and each of the etiological factors that are involved in its pathogenesis, immune maladaptation, placental ischemia, or oxidative stress may have a genetic implication [19].

4. The Genetic Component

From an epidemiological point of view, many studies have shown that PE is a disease with a strong familial predisposition, which also varies according to geographical, socioeconomic, and racial features. It has been reported that women with first-degree relatives with PE have 5 times more risk of developing the disease, while those with second-degree relatives have their risk doubled [20, 21]. Moreover, it is believed that paternal genes also play an important role in the development of PE. This is evidenced by the increased risk of PE in women with pregnancies of men who have previously been involved in pregnancies complicated with PE [22, 23]. This is of special importance since genomic imprinting results in involvement of paternal genes in the control of invasion and placental growth, whereas maternal genes inhibit it and are responsible for the adaptive immune response of pregnancy [24]. A large genetic association study of PE was published by Goddard et al. [25] that reported a study evaluating 775SNPs in 190 genes in more than 350 PE mother and offspring pairs and 600 control pairs. They detected six genes with a significative maternal-fetal genotype interaction related to PE in *IGF1*, *IL4R*, *IGF2R*, *GNB3*, *CSF1*, and *THBS4*. These findings and others suggest a multifactorial polygenic inheritance with a genetic component in the development of this disease [26, 27].

Table 1: Overview of the different polymorphism described.

Gene	Function	SNP	Reference
ERAP 1-2	Aminopeptidases 1-2. Related to immune antigen presentation	p.392 K>N and p.669L>Q	[28]
TNFSF13B	Regulation of immune response, member of tumor necrosis factor family	rs16972194 rs16972197 rs56124946	[29]
HLA-G	Member of HLA class I molecule and has immunosuppressive properties	G*0106 *HLA-G*	[30]
VEGF	Modulates the cell cycle, migration, and differentiation	rs1485766 rs6838834 rs7664413 rs2010963 +936 C>T	[31, 32]
Flt-1	FMS-related tyrosine kinase. It shows tyrosine protein kinase activity	rs12584067 rs7335588 rs722503	[31]
eNOS	Endothelial nitric oxide synthase	p.Glu298As-786T>C	[33]
CYP11B2	Steroid 11/18-beta-hydroxylase	−344C/T	[34, 35]
Prothrombin (F2)	Increases blood viscosity and the likelihood of thromboembolic events	G20210A	[36]
Factor V	Coagulation factor V	1691G>A	[36–38]
SERPINE1	Endothelial plasminogen activator inhibitor-1 (PAI-1), the major inhibitor of fibrinolysis	4 G/5 G polymorphism	[39]
MTHFR	Methylenetetrahydrofolate reductase	rs1801133 A1298C	[40–46]
MTRR	Methionine synthase reductase	rs1801394	[40–43, 46, 47]
MTR	Methyltetrahydrofolate-homocysteine S-methyltransferase	g.2756A>G	[48–50]

SNP: single nucleotide polymorphism.

Many biological candidate genes and polymorphisms have been examined in its relation with PE. We will discuss some of them, grouped by the different pathogenic mechanisms involved in PE (see Table 1).

4.1. Immune Maladaptation

4.1.1. ERAP1 and 2. The endoplasmic reticulum aminopeptidases 1 and 2 (ERAP1 and 2) are important in the immune response in terms of the antigen presentation [51] and they are colocalized within the endoplasmic reticulum (ER). The process starts with a proteolysis by the proteosome in the cytosol, and finally N-extended peptides are processed by aminopeptidase to mature the epitope which is presented by MHC class I. Data in mice have shown that ERAP1 trims MHC class I presented peptides in vivo and is the major trimming enzyme in the ER lumen; however ERAP2 also plays a role in the trimming of proteins [52]. In a murine model, it has been demonstrated that ERAP1 can be secreted in response to LPS/INFγ and activate macrophages in culture [53]. In human placenta, aminopeptidase RNA of ERAP1 has been detected by RT-PCR [54]. Recent studies in Australian/New Zealand and Norwegian populations have shown that *ERAP2* SNPs (p.392 K>N and p.669 L>Q; rs2549782 and rs17408150, resp.) are associated with PE susceptibility [28]. Recently, these SNPs have been also associated with increased risk of hypertensive disorders in pregnancy in African American population, but not in a Chilean one [55], supporting the idea that PE has a heterogeneous basis with variation between different ethnic group.

4.1.2. TNFSF13B. TNFSF13B is a member of the tumor necrosis factor family of ligands. *TNFSF13B* is localized in chromosome region 13q32-q34 and is implicated in the regulation of immune response to infections, autoimmune disease, and inflammation. In the third trimester of pregnancy, human placenta expresses TNFSF13B in villous cytotrophoblast cells (CT) and mesenchymal cells from villous core (MC). The receptors for TNFSF13B are expressed in both tissues [56, 57]. It has been proposed that the role of TNFSF13B [56] may be to modulate the immune system of the mother or to help in the development of fetal immune system. At the placental level, it is believed that TNFSF13B has an antiapoptotic effect [56]. Recently, Fenstand et al. [29] have shown a genetic variation of *TNFSF13B*, which is correlated with the susceptibility of PE. In his study, these authors detected three rare SNPs in *TNFSF13B* (rs16972194, rs16972197, and rs56124946) in Australian/New Zealand families with PE, but not in Norwegian ones. These results suggest that TNFSF13B could contribute to normal immunological adaptation during pregnancy, and the presence of these SNPs contributes to an abnormal placentation in some populations.

4.1.3. HLA-G Gene Polymorphism. This gene, localized in 6p21.3, is member of HLA class I molecule and has immunosuppressive properties. This characteristic has been implicated in the modulation of the maternal immune system, giving a possible inhibiting role during pregnancy when the mother gets in contact with the fetus [30]. HLA-G polymorphism is associated with recurrent spontaneous abortion and

PE. Moreau et al. [30] analyzed several polymorphisms in placenta of pregnant women with PE and controls, finding a higher frequency of G*0106 HLA-G polymorphism in preeclamptic placentas compared with control (21.2% and 6.6%, resp.), suggesting a modulator effect of *HLA-G* over pregnancy and the risk of PE.

4.2. Vascular and Endothelial Function

4.2.1. VEGF. The VEGF is a family with seven members, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and PIGF. VEGF-C is expressed mainly in the heart, placenta, ovary and embryonic tissues [58]. The receptors of VEGF named VEGFR-1/Flt-1, VEGFR-2/Flk-1 and VEGFR-3/Flt-4 are VEGF tyrosine kinase receptors. VEGFR-1 has a much weaker kinase activity and modulates the endothelial cell cycle. VEGFR-2 is the more important receptor for VEGF in VEGF-induced mitogenesis and permeability. VEGFR-3 is expressed in lymphatic endothelial cells and participates in mitosis, migration, differentiation, and survival cells [58]. Several polymorphisms in VEGF have been correlated with an increased risk of PE. Polymorphisms in VEGF-A have been implicated in the risk of PE, and down regulation of VEGF and VEGFR expression has been also reported in patients with severe PE [59, 60].

A recent study using an array for 50,000 genecentric SNPs identified 124 SNPs in 6 genes related to angiogenesis. This data indentified allelic variation of *VEGF-C*, *Flt-1*, and *Flt-4* from 606 women (489 African American and 117 white Caucasian). Two *VEGF-C* SNPs (rs1485766 and rs6838834) were associated with PE in African American women and one *VEGF-C* SNP (rs7664413) was associated with PE in Caucasian women [31]. This author also highlights the association between *Flt-1* polymorphism and PE, showing that two *Flt-1* SNPs (rs12584067 and rs7335588) are associated with PE in black women and other two, *Flt-1* SNP (rs722503) and *Flt-4* (rs307826), are more prevalent in white patients with PE [31]. However, some data have shown that some polymorphism of VEGF can be protective for PE. In a Hungarian cohort of nulliparous patients, a study looking for the *VEGF* r. 405 C > polymorphism has found that patients with the *VEGF* 405 G allele have less severity of PE [61]. Nevertheless, a recent study [62] showed that the *VEGF* 405 G allele (rs2010963) is not associated with PE in a Mexican population of pregnant women. These differences highlight the variability and implications of the presence of some SNPs in diverse ethnic groups.

Finally, Shim et al. [32] have characterized another polymorphism of *VEGF* in Korean pregnant women using PCR and restriction fragment length polymorphism assay. The *VEGF* +936 C>T has been located in the 3′-unstranslated region (UTR), and it has been associated with PE. However, the small number of patients in this study precludes making more general conclusions.

4.2.2. eNOS. The NO is synthesized by endothelial nitric oxide synthase (eNOS, NOS3) using L-arginine as a substrate. NO is the endothelium-derived relaxing factor and has a crucial role in the regulation of smooth muscle tone in the vascular system [63], being a critical element for the correct blood perfusion of the placenta. Recently, it has been reported that the activity of eNOS is reduced in patients with PE [33]. Several polymorphisms have been described for eNOS, but two of them have been strongly related with PE, SNP G c.894 G>T, which encodes an amino acid substitution in eNOS (p.Glu298Asp) and −786 T>C polymorphism. A study in 844 Colombian pregnant women reported that patients homozygous for Asp298 (894 T) allele have more risk of PE than women with Glu298 allele [64]. Similarly, an important correlation has been reported between hypertension and Asp298 genotype in pregnant women of Japan [65].

4.2.3. CYP11B2. The steroid 11/18-beta-hydroxylase is encoded by *CYP11B2* gene that is located in 8q24.3. The protein is physically localized in mitochondrias of the zone glomerulosa of the adrenal cortex, synthesizing the mineralocorticoid aldosterone [66]. The −344 C/T polymorphism within 5′regulatory region of *CYP11B2* disrupts a putative steroidogenic factor-1 site, and the homozygosity for SF-1 T/T variant has been reported as a protective factor against the risk of PE. In contrast, the heterozygous state is not protective of PE [34]. Moreover, −344 C/T polymorphism has a strong linkage disequilibrium respect intron 2 polymorphisms in *CYP11B2* and contributes to hypertension in subjects with a raised aldosterone-to-renin ratio [35].

4.3. Thrombophilic Disorders.

Thrombophilic conditions are associated with an increased risk of venous thromboembolic events during pregnancy; however, there is still controversy as to whether they may adversely affect other pregnancy outcomes such as PE [67]. Women with PE have levels outside the normal range for fibronectin and von Willebrand factor, that are markers of endothelial cell injury [68], and recently several polymorphisms of genes coding for vascular proteins have been associated with PE.

4.3.1. The human Prothrombin (F2). The gene is localized in chromosome 11p11-q12, and the increase of prothrombin during pregnancy can neutralize the effect of physiologic hemodilution, increasing blood viscosity and the likelihood of thromboembolic events [68]. Recently Seremak-Mrozikiewicsz et al. [36] have shown in Polish population that a polymorphism of prothrombin (G20210A) could be associated with an increased risk of developing severe PE.

4.3.2. Factor V Gene. The gene is localized in the 1q23 implicated in several diseases. Several studies, have suggested an important correlation between factor V Leiden SNPs (1691 G>A) and the risk of severe PE [37, 38] and a recent meta-analysis has confirmed these findings [69]. The study of Seremak-Mrozikiewicsz [36] also showed that 1691 G>A polymorphism contained at least one variant allele A (GA and AA) in the group of women with severe and mild PE.

4.3.3. SERPINE1 Gene. PE is associated with thrombosis of the intervillous space of the placenta. The *SERPINE1* gene encodes endothelial plasminogen activator inhibitor-1 (PAI-1), the major inhibitor of fibrinolysis (member of the serine protease inhibitor family). Yamada et al. [39] assessed the association between PE and the 4 G/5 G polymorphism of the *PAI1* gene in 115 PE patients, 210 pregnant controls, and 298 healthy volunteer controls. The frequency of homozygotes for the 4 G allele was significantly higher in the patients than in the control pregnant women or healthy volunteers. The 4 G allele frequency was also significantly higher in patients than in the control groups. However, other studies have not found the same association [19].

4.4. Metabolism and Oxidative Stress

4.4.1. PON-1. The paraoxonase-1 (*PON-1*) is a member of a three-gene family (*PON-2* and *PON-3*), and is located in chromosome 7 (q21.22). The protein, primarily synthesized in the liver, has a release into the circulation and is associated with high-density lipoproteins (HDLs). The principal action of PON-1 is the protection of acute toxicity and oxidative stress involved in the development of atherosclerosis. PON-1 also has a role in the hydrolysis of organophosphate insecticides and nerve agents [70, 71]. There is evidence that shows that preeclamptic women have lower levels of serum HDL, higher level of serum triglycerides, and lower level of serum Apo-A1 than control women [72], and this has been correlated with lower PON-1 serum activity [73]. A study in 3266 Caucasian women who were randomly selected from 23 British towns investigated the relation between *PON-1* p.Q192R polymorphism and hypertension. They did not find evidence of an increased risk of hypertension during pregnancy in carrier patients, but an association was seen with preterm birth. This polymorphism could reduce the hydrolysis and modify the redox equilibrium in the maternal blood [74]. A relation between *Pon-1* p.Q192R and serum oxidized LDL levels has been also reported [72]. Finally, Isbilen et al. [75] reported a differential distribution of *PON-1* p.Q192R and L55M polymorphism between PE and normal patients. The authors detected high levels of homocysteine concentration in preeclamptic women when homozygotes for 192RR and 55RR were present.

Iron-induced oxidation and free radical attack of the R-SH group of different maternal plasma molecules like to aminothiols, including cysteine, homocysteine, and cysteinylglycine result in the formation of radical disulfides. These changes produce disequilibrium in the redox thiol status, which is displaced to a higher oxidized state [17]. The level of maternal plasma homocysteine is higher in severe PE than in mild PE and control groups. Elevated levels of maternal homocysteine increase the risk for endothelial dysfunction and atherosclerosis and occlusive vascular disorders [76].

4.5. MTHFR, MTRR, and MTR Genetic Polymorphisms. Decrease in MTHFR protein levels or activity by different gene variants induces increased levels of homocysteine [40–42]. The most extensively studied variant in *MTHFR* gene is the polymorphism *MTHFR* g.677C>T (rs1801133), which changes an alanine to valine in aminoacid 222 (p.A222V) in the enzyme regulatory domain and causes a thermolabile enzyme with decreased activity at 37°C and so hyperhomocysteinemia [40–43]. This reduced activity of *MTHFR* due to *MTHFR* g.677C>T polymorphism may impair the remethylation pathway [44]. The frequencies for the T-allele/TT-genotype of *MTHFR* g.677C>T polymorphism are variable in different populations, being 0,11/0,00 in African Americans and 0,59/0,35 in Mexicans, respectively [45]. *MTHFR* A1298C is another common polymorphism and it produces the change of glutamate to alanine in aminoacid 429 (Glu429Ala) within the enzyme catalytic domain [40, 46, 47]. In general, the frequency of CC genotype is about 0.1 and C allele frequency is about 0.36 [42]. As with *MTHFR* g.677C>T, *MTHFR* A1298C polymorphism has been reported to be associated with altered methionine-homocysteine metabolism (MHM) and increased levels of homocysteine [41]. Polymorphisms in *MTHFR* gene and hyperhomocysteinemia have been associated with recurrent pregnancy loss, gestational hypertension, placental abruption and PE [40, 43].

The most common polymorphism in the *MTRR* gene is g.66A>G (rs1801394) substitution, changing isoleucine to methionine in aminoacid 22 (I22M). *MTRR* g.66A>G polymorphism has been associated with reduced activity of MTRR enzyme resulting in hyperhomocysteinemia and altering the methylation of DNA [42, 43, 47]. It has also been observed that the coexistence of the *MTHFR* g.677C>T genotype with the *MTRR* g.66A>G polymorphism may exacerbate the effect of the *MTHFR* variant alone [46].

A common polymorphism in *MTR* is g.2756A>G substitution, which results in an amino acid change of an aspartic acid to a glycine (p.D919 G), at the penultimate position in a long helix that leads out of the cobalamin domain. Having the glycine residue at this position could have an effect on the secondary structure of the protein and therefore have functional consequence [48–50]. Recently, Furness et al. [50] have evaluated one-carbon metabolism enzyme polymorphisms in patients with uteroplacental dysfunction, suggesting that the maternal and fetal *MTR* g.2756A>G allele represents an important risk factor for the development of uteroplacental insufficiency, which includes PE and intrauterine growth restriction [50]. The potential negative effect of combined polymorphisms of the *MTHFR*, *MTR*, and *MTRR* genes on plasma homocysteine levels in at-risk population needs further investigation.

5. Conclusion

PE is a common disease in pregnancy worldwide, causing substantial short- and long-term morbidity for the newborn and the mother. PE is a multifactorial disease, where maternal and fetal factors converge to result in a multicomponent risk. No single factor has been identified as capable of determining the disease, and several are needed to trigger symptoms of PE.

Various studies in different populations have identified maternal polymorphisms associated with PE through

candidate gene approaches. These findings will need to be complemented by currently available genome-wide approaches, evaluation of interaction between genes, genes and environment, and the contribution of paternal and embryonic genotypes. Further prospective studies will be necessary to assess the predictive potential of markers identified through these and other strategies.

Despite substantial advances in understanding the pathogenesis of PE, the development of simple tests to identify individuals or populations at risk still remains a substantial research, epidemiologic, and clinical challenge.

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