CEOG Clinical and Experimental Obstetrics & Gynecology

Review Article

Pre-eclampsia and the vascular endothelial growth factor: a new aspect

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Summary

Pre-eclampsia (PE) is a multi-system disorder of human gestation characterized by hypertension, proteinuria, and edema, which resolves with placental delivery. This disease affects 3-14% of all pregnancies worldwide and 5-8% in the USA. Furthermore PE remains one of the leading causes of maternal and neonatal mortality and morbidity worldwide. One of the most important goals in obstetrics is the early identification of the patient with an increased risk for PE. This paper unifies the essential and validated findings of past and current scientific investigation which encompass the relationship between PE and the vascular endothelial growth factor (VEGF). VEGF and its receptors have acquired great interest due to their vital role in neovascularization (vasculogenesis and angiogenesis) in a variety of physical and pathological processes such as the female reproductive cycle, PE, and tumorigenesis. VEGF is secreted in response to tissue hypoxia and endothelial cell damage. Alterations in the circulating levels of this factor may therefore identify those pregnancies with a high possibility of developing PE. This review will summarize the present authors' current understanding of the role of circulating VEGF in the pathogenesis, clinical diagnosis, and prediction of PE.

Key words: Pre-eclampsia; Hypertension; Gestational hypertension; VEGF; Pregnancy; Cytotrophoblast.

Introduction

Pre-eclampsia (PE) is the hypertensive disorder of pregnancy, presented with two significant phenotypes, high blood pressure, and proteinuria after 20 weeks' of gestation [1, 2]; in multiple gestation and molar pregnancy, PE can also manifest itself in the same clinical type before the 20th week. PE is divided into preclinical and clinical stages; the term comes from the Greek word "lighting". It is the final stage prior to eclampsia, a state characterized by generalized grand–mal seizures [3-6]. The disease is so-named as the precursor to eclampsia, whereas the child-bearer experiences a new onset generalized grand mal seizure; that is the novel seizure manifestation. The preclinical part is characterized by abnormal placentation before the 20th week of gestation; the clinical part is marked by elevated blood pressure, proteinuria, inadequate cytotrophoblast invasion, and systemic endothelial dysfunction after the 20th week [7-10].

The diagnostic criteria include the following: systolic blood pressure >140 mmHg or diastolic blood pressure > 90 mmHg and proteinuria defined as urinary excretion of ≥ 0.3 grams in 24-hour collection with no evidence of urinary tract infection [11, 12]. The disease is characterized by visual disturbances, edema, seizures, headaches, epigastric pain or right upper quadrant pain, impaired hepatic function, hemolytic anemia, elevated liver enzymes, low platelet count (HELLP syndrome), and intrauterine growth restriction. In addition to elevated blood pressure and proteinuria levels, severe PE is based on the following criteria: oliguria (< 500 ml in a 24-hour sample), cerebral hemorrhage, visual disturbances, pulmonary edema, and liver rupture [11, 13, 14]. Eclampsia can be complicated by placental abruption, renal failure, subcapsular hepatic hematoma, preterm delivery, convulsions, cerebral hemorrhage, and even fetal or maternal demise [11, 13].

Placental tissue is necessary for the evolvement of PE. Removal of the placenta after delivery converts the maternal state back to normal; the syndrome may persist if placental tissue is retained. These observations have led investigators to consider that there is perhaps a circulating factor or factors produced by the placenta contributing to maternal disease.

Pregnancy is thought to be a cardiovascular stress test; it can be a tool of detecting women with an increased risk of developing vascular disease. Risk factors such as hypertension, diabetes, obesity, a previous history of PE and the presence of coronary artery disease (CAD) increase the risk for PE, leading to severe early-onset disease with greater

Revised manuscript accepted for publication October 22, 2014

Clin. Exp. Obstet. Gynecol. - ISSN: 0390-6663
XLIII, n. 1, 2016
doi: 10.12891/ceog2057.2016

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frequency and also with adverse neonatal outcomes [7, 18]. Efficient placental function guarantees the normal exchange of nutrients between the fetus and maternal side [7, 15]. The under-perfused placental mass releases a variety of placental factors into the maternal circulation affecting vascular spurting, growth and permeability, and endothelial dysfunction [15-18]. Impaired trophoblastic invasion of the maternal placental area combined with the imbalance between the angiogenic factors are considered to be the initiation steps of endothelial dysfunction in PE, resulting in impaired uteroplacental blood flow and subsequent local placental hypoxia [8, 18, 19].

VEGF-polymorphisms of VEGF

VEGF is the essential molecule secreted by the cytotrophoblasts and guarantees the vasculature stabilization in the human organism. It is a heparin–binding, dimeric glycoprotein of 45,000 daltons member of the platelet derived growth factor (PDGF) family of mitogens with potent angiogenic properties secreted in response to tissue hypoxia and endothelial cell damage. It acts through transmembrane tyrosine kinase receptors on vascular endothelial cells. VEGF exists in four molecular types which have respectably 121, 165, 189, and 206 amino acids. VEGF is a cytokine molecule, essential for endothelial integrity and stabilization with vasodilative properties; it induces nitric oxide and prostacyclin synthesis by endothelial cells, endothelial cell proliferation, and migration. The two main VEGF receptors are the Flk-1 (VEGFR-2) and the Flt-1 (VEGFR-1). The interaction of VEGF and placental growth factor (PIGF) with their endogenous receptors is prevented by sFlt-1 which is located on monocytes. In PE pregnancies we anticipate the presence of oxidative stress, systemic inflammation, and fluctuations in the circulating levels of the angiogenic factor. [2].

The crucial role of physiological vasculogenesis and vascular permeability is assigned to VEGF. VEGF is known to be a useful marker of early vascular development. The location of the gene encoding VEGF is on chromosome 6 band p21 and comprises a 14 kb coding region with eight exons and seven introns. The chromosome's VEGF family displays different biological activities, due to their different specificities for the known receptors. Its properties are exerted through the existence of the two high-affinity tyrosine kinase receptors, Flt-1 (also known as VEGFR-1) and kinase insert domain receptor (KDR in humans)/(Flk-1 in mice), (also known as VEGFR-2); a membrane and a soluble isoform compose both receptors [9, 11, 12, 20-22]. In several studies in both animals and humans, the effect of blockage on VEGF action has been suggested to be the milestone of the pathophysiology of PE. VEGF signaling is critical for the establishment and maintenance of the glomerular filtration barrier; in this concept, anti-VEGF therapy produces proteinuria. It is possible that decreased VEGF activity produces the renal features of PE. In a wide range of renal diseases, the deregulation of VEGF expression has been demonstrated within the glomerulus. Therefore, we would expect that VEGF polymorphisms might affect the occurrence of PE [17].

Clinical studies in women have shown that circulating total VEGF concentrations are significantly decreased in women with PE. VEGF is thought to be involved in the pathogenesis of PE rather than being an effect of the disease. Genetic polymorphisms on VEGF could affect the susceptibility to the development of PE or gestational hypertension (GH); this is seen in relevance to VEGF in normal pregnancy; the abnormalities in VEGF function are possibly associated with PE or GH. Family studies have shown that genetic factors might play a role in PE; however the exact inheritance pattern is still unknown. A few of them have been correlated with variation in VEGF protein production [17]. Therefore, PE is characterized by normal to high total VEGF levels (probably induced by placental hypoxia) but low free VEGF levels, owing to a vast excess of sFlt-1 which antagonizes the VEGF effects on the formation of placental vasculature and maternal endothelial cell function. A second trimester analysis of circulating VEGF appears to be a useful tool for the early identification of pregnant women who are at increased risk for developing severe, early onset PE; measuring VEGF levels in the umbilical vein and artery and investigating maternal and fetal VEGF polymorphisms is informative regarding the possible associations between VEGF and PE [20].

Taking into account the essential role of VEGF in pregnancy, polymorphisms of the VEGF gene were assumed to be important markers to determine the liability to PE. Based on genetics, this interaction delineates the association between genetic polymorphisms of VEGF and an increased risk of developing PE [14].

Genetic polymorphisms of the VEGF gene, linked to an inherited alteration of VEGF production, may contribute to the pathogenesis of PE. The carrier state of the $VEGF^{+405G}$ allele, which is accompanied by high VEGF-producing capability, decreases the risk of severe PE. An inherited ability of VEGF secretion could be protective against PE in relation to earlier clinical experience that VEGF has a potential effect on placentation [13].

In conclusion, extensive genetic studies should be launched to improve statistical accuracy, to explore the functional applicability of VEGF polymorphisms and their relationship with PE [14].

The pathophysiology of PE

Normal pregnancy is associated with an increased endothelium-induced immediate response to angiotensin II, epinephrine, and increased blood flow [2, 23]. Blood flow to the uterus increases approximately tenfold in gestation, half of which goes to the placental unit and the fetus. Studies in

various animal models have shown that reduction in uteroplacental blood flow can lead to a hypertensive state that closely resembles PE [2, 7, 24]. The ischemic and poorly perfused placenta is thought to be the basis of the underlying pathology of PE [24]. PE originates in the placenta starting with inadequate cytotrophoblast invasion into the uterine spiral arteries and ending in widespread maternal endothelial dysfunction. PE is divided in three stages: abnormal remodeling of the placental bed vascular, placental ischemia, and dysfunction of the endothelial cells' layer. Pathological development of the placental vessels results from insufficient trophoblast invasion of maternal spiral arteries during the first days of gestation. Abnormalities in trophoblast invasion and generalized maternal endothelial dysfunction seen in PE may be triggered via release of placental factors. Pre-eclamptic women demonstrate significantly increased levels of fibronectin, the Von-Willenbrand factor, decreased NO production, increased ratio of thromboxane/prostacyclin; compounds that comprise the serum markers of endothelial cell injury [19, 23]. The concentration of serum markers of endothelial cell injury and abnormal placental development are reflected in maternal circulation [2]. The hypoxic and poorly perfused placenta induces a systemic inflammatory response that results in an altered vascular function by trophoblasts; the trophoblasts replace the endothelial cells of the spiral arteries. As a result the placental vasculature converts from a large-caliber to a low-capacitance high blood flow vascular network [3, 24, 25]. There is restriction of blood flow into the intervillous space leading to placental hypoxia. Placental ischemia leads to production of soluble factors that can cause maternal endothelial dysfunction. Under hypoxic conditions, the endothelial and neoplastic cells express proteins known as endothelin, 1L-1a, and VEGF; these proteins invade the surrounding tissues [17, 26]. Hypoxia also initiates the expression of PDGF mRNA and VEGF mRNA in tissue cultures indicating that oxygen is an important angiogenesis regulator [25-27].

Additional clinical studies have shown, the syncytiotrophoblast, the villous cytotrophoblast, and the fetoplacental vasculature expressing the HIF-1a and HIF-2a factors; they are present in pre-eclamptic placentas of any gestational age. HIF-2a is strongly expressed in pre-eclamptic placental tissue and not down-regulated upon oxygenation [28].

Clinical studies revealed, that female subjects with signs of PE and those giving birth to small-for-gestational-age (SGA) newborns show elevated plasma levels of the soluble form of the VEGFR-1 and the soluble form of the Endoglin (s-Eng) molecules; VEGF and PIGF are low in concentrations in the same subjects [16, 27, 28]. As a consequence of placental hypoxia, there is production of sFlt-1 in PE, which is related to the failure of the cytotrophoblasts invading into the spiral arterioles. Decreased oxygen tension of normal villous explants cause a two-fold-elevation in sFlt-1 secretion level. Before the occurrence of PE, PIGF serum level is markedly decreased.

The PE syndrome follows secondary to aberrant placentation and excess placental secretion of sFlt-1. The starting point of PE is the placental mass; the placenta undergoes the pathways of vasculogenesis and angiogenesis; angiogenesis is the process of neovascularization from pre-existing blood vessels, whereas vasculogenesis is the process of blood vessel generation originating from the angioblast precursor cell. During fetal development, the human placenta is characterized by increased activity of angiogenesis and vasculogenesis. [3, 13, 23]. Delivery of the placenta allows the hemodynamic parameter of blood pressure to return to a normal level. Severe PE is related to placental hypoperfusion and ischemia; placental infarcts, acute atherosis, fibrin deposition, vascular intimal thickening, and endothelial damage can be detected. Uterine artery Doppler abnormalities are suggestive of increased impedance to blood flow in the uterine circulation and failure of the physiologic transformation of the spiral arteries, diagnosed through histological examination of the placenta [5, 10, 19].

As it has already been noted, during the early first steps of normal placental growth, fetal extra-villous cytotrophoblasts invade the uterine spiral arteries of the decidual superficial layer and the myometrium. Maternal spiral arteries are being transformed from small high–resistance vessels to large diameter capacitance blood vessels, having the ability to provide adequate blood perfusion to support fetal growth; in PE, this procedure is incomplete [29].

The role of angiogenic and antiangiogenic factors in placental vascular development

Angiogenic factors are the main contributors to placental vascular development; moreover their receptors are of great significance for normal placentation and embryo development [24, 30]. Expression studies show that the growth of the human placenta is related to angiogenic growth factors and their receptors. Hybridization and immunohistochemical studies of VEGFRs on the human placenta demonstrate that the receptors are localized in the villous trophoblast and macrophages of both fetal and maternal origin [17].

PIGF and VEGFR-1 (Flt-1) are being expressed in the trophoblasts; expression of these proteins is altered in PE. The main regulator of angiogenesis is sFlt-1, which is produced by splicing of the VEGF mRNA. The binding process of the VEGF receptors (VEGFR-1 and VEGFR-2) is prevented by sFlt-1 which causes low circulating levels of free VEGF, and PIGF [2, 11, 12, 16, 27, 28, 30]. VEGF and PIGF are produced in excessive concentrations in the trophoblastic villi in gestation and inactivated by sFlt-1; cytotrophoblasts are prevented from spreading out and developing in utero by the sFlt-1 molecule. The titer of sFlt-1 remains low during early gestation and reaches a maximum in the late third trimester; a reason why this biomarker cannot be used efficiently in the diagnosis of PE [17]. There is enhancement of the sFlt-1 molecule during the late third

trimester in the maternal circulation which increases with Eng. This may lead to maternal endothelial dysfunction and the clinical syndrome of PE.

The pathogenesis of PE is the result of the imbalance of the angiogenic and anti-angiogenic factors; there is an increased expression of soluble fms-like tyrosine kinase-1 (sFlt-1) and a decreased PIGF and VEGF expression profile [9, 10, 12, 16, 27, 31]. In experiments with pregnant rats it has been observed that vessel injection of sFlt-1 causes proteinuria and hypertension [32]. The structural integrity of the glomerular ultrastructure and the fenestration of the glomerular endothelial cells is an important biological property for VEGF [33].

Eng is the co-receptor for the transforming growth factors - β1 and -β3 located on the endothelial cells; it increases vascular permeability and induces high blood pressure in rodents. It has been observed that there are increased levels of the Eng molecule two to three months before the onset of PE. The molecules, s-Eng and sFlt-1 might lead to endothelial dysfunction and the syndrome of PE [2, 30]. Furthermore, the addition of sFlt-1 in pregnant rats results in the classical pre-eclamptic triad of hypertension, proteinuria, and renal endotheliosis [34].

It has also been observed that sFlt-1 and Eng show potent anti-angiogenic properties [12]. In addition s-Eng binds and antagonizes TGF-b (TGFb-1 and TGFb-3); it is upregulated in PE and is highly expressed in syncytiotrophoblasts and the cytotrophoblasts. In pre-eclamptic women, the s-Eng molecule is elevated in the blood at eight to 12 weeks before the clinical outbreak of the disease [17, 23, 26, 30]. s-Eng is a co-receptor detected in endothelial cells and the syncytiotrophoblasts. The genetic mutations on the Eng molecule can be the result of the loss of the capillary number and occurrence of multiple arteriovenous malformations [6].

SFlt-1 increases its serum concentration in pre-eclamptic females five to ten weeks before the onset of the disease, with concurrent serum fall of the free VEGF and PIGF titers, which are expressed in both bovine maternal and fetal tissues. This expression in relation with their receptors, VEGFR-1 and VEGFR-2, increases through the period of maximal placenta development [11].

Conclusions and future directions

Invasion of the uterine wall by fetal extravillous trophoblastic cell is the triggering event in PE. The clinical features associated with PE can be initiated by placental factors which enter the maternal circulation and cause endothelial dysfunction resulting in hypertension and proteinuria. Ischemia or hypoxia of the placenta forms the basis of PE, resulting from defective progression of the spiral arteries' remodeling and placental angiogenesis. In women with PE, studies have revealed that the total VEGF titer is significantly elevated. In pre-eclamptic subjects the biologically active free VEGF concentration was decreased and the sFlt-1 concentration was elevated. The specific mechanisms which lead to excess sFlt-1 production by the placenta, the role that sFlt-1 plays in normal development and PE, the relation between the sFlt-1, PIGF, and VEGF factors in PE are still unknown. PE is multifactorial with an onset, severity, and progression that can be significantly different among child-bearers. There is no definitive predictive test available to target women who will develop PE. New prospects have been introduced by recent advances in the understanding of PE and the identification of women who are liable to manifest the disease. The regulation of angiogenic gene products and their role in placental angiogenesis and systemic vascular health compose the new aspect of pre-eclamptic therapeutic and diagnostic options. The majority of women who develop PE demonstrate the whole range of the clinical manifestations of the PE syndrome. The fact that many of the proposed biomarkers for PE are increased in smaller concentrations in normal pregnancy makes the identification of accurate biomarkers for PE more difficult. If signs of abnormal placental and endothelial dysfunction could be detected prior to the onset of the clinical disease, this would represent an extremely attractive field for emerging therapeutic strategies. Soluble angiogenic markers used either alone or in a combination with other markers would strongly encourage research in the diagnosis and screening of PE. It is hoped that one or more of the various markers associated with PE will prove useful as a potential screening tool to identify those women destined to develop PE in pregnancy. Early diagnosis gives patients an opportunity for an early and effective remedy. The development of new drugs that would be effective in treating or preventing this devastating disease could begin with the identification of circulating angiostatic factors such as sFlt-1.

Acknowledgments

This work was supported by Democritus University in Alexandroupolis in Greece.

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