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Review article

Role of growth factors in preeclampsia: Early detection and treatment

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ABSTRACT

Preeclampsia is a pregnancy specific condition characterized by hypertension and proteinuria. It complicates about 10% of all pregnancies. It is a major cause of maternal and fetal morbidity and mortality. Interestingly, preeclampsia may have an impact on the health of the mother or infant, beyond the pregnancy. It is believed that several ligands and receptors of different families of growth factors have been involved in the development of preeclampsia. We performed a systematic search of PubMed including combination of terms such as preeclampsia, growth factors, treatment, vascular endothelial growth factor A, activin A, inhibin A, placental growth factor, transforming growth factor β-1, Nodal, placenta, trophoblast cells, biomarkers and detection. In this review we have summarized current knowledge on the role of growth factors in early detection and treatment of preeclampsia. Although these growth factors have significant roles in normal and complicated pregnancies, the current value of these growth factors as biomarkers, for the precise prediction of preeclampsia, has its limitation. Therefore, future studies need to be done to support some of the very promising and interesting data to develop affordable and widely available tests for early detection and treatment of preeclampsia.

Keywords: preeclampsia, growth factors, placenta, bio markers, treatment, detection, vascular endothelial growth factor A, activin A, inhibin A, placental growth factor, transforming growth factor β-1, Nodal

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INTRODUCTION

Preeclampsia is the contributor to the world wide maternal mortality of approximately 100,000 deaths a year.[1] It complicates about 10% of all pregnancies and it is the first cause of maternal admission to intensive care units.[2] It is a very challenging disease with associated high risk of perinatal mortality; it is the reported cause of 10% stillbirths and 15% of preterm deliveries.[1,3] There is a five folds increase in the death rate of infants born to mothers affected with preeclampsia. In a retroscopic study involving one million Canadian women, it was shown that women, who suffer from preeclampsia or other placental diseases, are twice as likely to have premature cardiovascular disease when compared to women with no placental syndrome.[3–6]

No recent data is reported about the prevalence of preeclampsia in Qatar. However, in a population-based study carried out in UAE, preeclampsia was the leading cause of maternal morbidity. It has been recently reported that 59.5% women out of 926 cases of severe acute maternal morbidity were preeclamptic. The frequency of preeclampsia was significantly higher among Indian subcontinent populations as compared to Arab populations.[7]

Preeclampsia is a pregnancy-specific condition characterized by hypertension (≥140/90 mm Hg) and protienuria (≥300 mg) after 20 weeks of gestation. Clinical symptoms of preeclampsia include headaches, excess and rapid weight gain, nausea and vomiting, stomach ache, edema and vision problems.[8] If left untreated, preeclampsia can develop into eclampisa, which is acute and life threatening occurrence of seizure activity and/or unexplained coma during pregnancy or postpartum.[9] Preeclampsia may also impact women's health beyond their pregnancies. Increasing evidence suggest that preeclampsia is the risk factor of many diseases including cardiovascular diseases and diabetes.[4–6,10]

Two different disease entities of preeclampsia are described based on number of articles published in early 1980s and late 1970s: early onset preeclampsia (that develops before 34 weeks of gestation) and late onset preeclampsia (that develop at or after 34 weeks of gestation).[3,11–15] Different genetic and environmental risk factors, prognosis, heritability, biochemical, histological and clinical features are associated with early or late onset of preeclampsia. Early onset preeclampsia is associated with dysfunction in placenta, reduction in placental volume, intrauterine growth restriction, low birth weight and adverse maternal and fetal outcomes. On the other hand, in general, placental involvement is minimally present in late onset preeclampsia and is the result of maternal constitutional disorder. Furthermore, normal fetal growth, normal birth weight and favourable maternal and fetal outcomes have also been reported in late onset preeclampsia.[16,17]

Predicting preeclampsia is a major challenge in obstetrics.[17,18] More importantly, no major progress has been achieved in the treatment of preeclampsia. As the placenta is the main cause of the disease, the only way to treat the disease is to extract the placenta and deliver the baby. In developed countries, the cost of an average case of preeclampsia is estimated at £9000.[19] In developing countries, where emergency care is often inadequate or lacking, the importance of preeclampsia is even more stressed, when confidential enquiries are analysed showing that a significant proportion of cases of fetal deaths is due to preeclampsia.[20] Therefore, there is an increasing need of an affordable and widely applicable test that could permit early presymptomatic detection of preeclampsia to identify and monitor high-risk pregnant mothers to provide the best prenatal care for them and for their child.

There are multiple markers of preeclampsia that are available in the first trimester and would allow early diagnosis of high-risk pregnant women to reduce the morbidity. These markers can also open an area to carry out further studies looking at therapeutic medications.[12,14,18,20–24]

In this review we will briefly discuss the pathophysiology of preeclampsia, current biomarkers in preeclampsia and prospects of use of these markers for early detection and treatment of preeclampsia.

PATHOPHYSIOLOGY OF PREECLAMPSIA

Preeclampsia is a multisystem disorder commonly called as gestational hypertension with protienuria or defective placental angiogenesis.[3,5,12,16,25] It is the leading cause of maternal and fetal mobidity and mortality. Prediction of development of preeclampsia is often too difficult, leading the Greeks to name 'eklampsis' means lightening.[3] Severity of preeclampsia is variable. It ranges from mild hypertension and proteinuria to severe disease with endothelial dysfunction and end organ damage. Preeclampsia is investigated for more than a decade now, but most of its pathogenesis is still unknown.[26]

Increasing evidence suggest that preeclampsia is the result of placental dysfunction in the first trimester. This placental dysfunction is because of the shallow invasion of extravillous trophoblast cells in the placental bed.[11,17]

Placenta is a transient organ, which is critically involved in the growth and development of the fetus. During placental development, trophoblast is the first cell lineage, differentiated into two major pathways, the villous pathway and extravillous pathway (Figure 1). In normal pregnancy, during the first trimester, extravillous trophoblast cells invade the maternal spiral arteries of the uterus, into and across the deciduas up to the one third of the myometrium.[27] This results in the formation of maternal placental vessels that supply oxygen and nutrients to the developing fetus. Trophoblast cells also abolish the response of maternal vessels to the vasoconstrictors by clearing the smooth muscle structure that surrounds these vessels, therefore facilitating placental blood flow. On the other hand, in preeclampsia, invasion of myometrium by extravillous trophoblast is reduced to around 50% of normal, resulting in incomplete modification of maternal spiral arteries, placental ischemia and hypoxia which impairs fetal growth (Figure 1).[27–29] Shallow invasion of the uterus also produces a

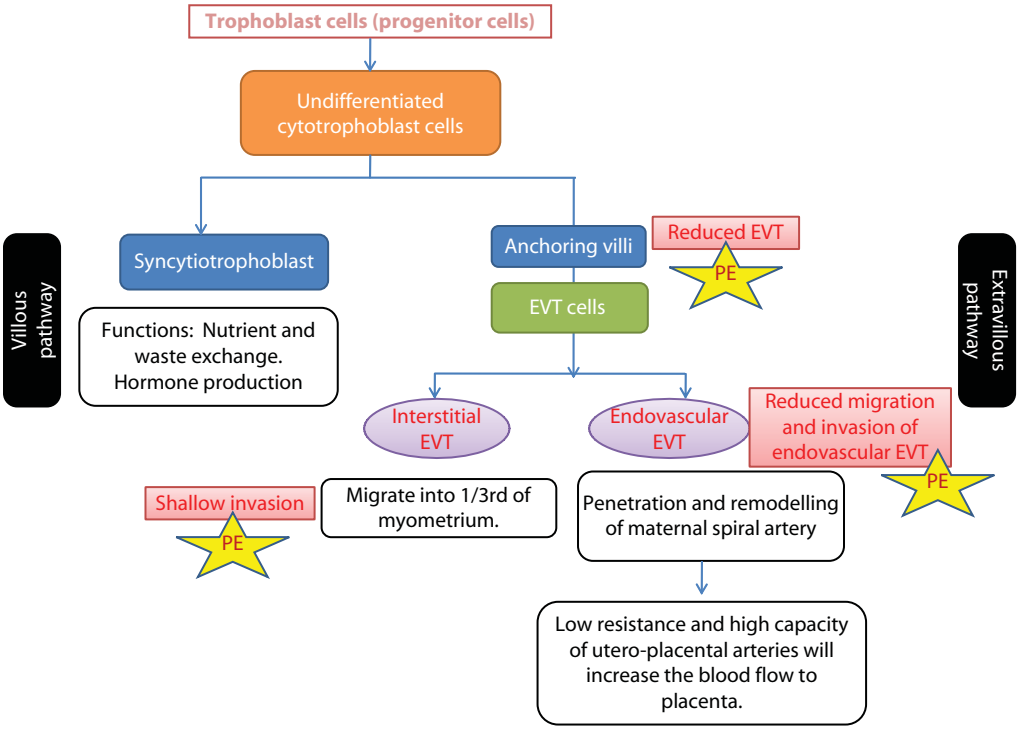

Figure 1. A schematic flow chart to illustrate pathways of normal placental trophoblast differentiation vs. preeclampsia formation. Trophoblast progenitor cells give rise to cytotrophoblast, which are mononucleated and undifferentiated cells. Cytotrophoblasts differentiate into multinucleated syncytiotrophoblasts, in the villous pathway. In the extravillous pathway, cytotrophoblast cells detach from the anchoring villi, called Extravillour trophoblast cells (EVT) and migrate into the decidua. These cells reach up to one third of the myometrium and are called interstitial extravillous trophoblast cells. Endovascular extravillous trophoblast cells penetrate the uterine spiral artery and causes remodelling of spiral artery to increase the blood flow in placenta. Red coloured boxes indicate pathological changes in normal pregnancy that leads to preeclampsia.

series of modulators of angiogenesis by the placenta which crosses the materno-placental barrier and adversely affects the mother in the later stages of pregnancy. Thus, the response by the mother appears late, the origin of preeclampsia is early, local and placental.[5,6] Several growth factors are involved in the normal placental development. The functions, location and expression of these growth factors are very important in the regulation of placental development, and are thoroughly reviewed in various studies (Table 1).

CURRENT BIOMARKERS IN PREECLAMPSIA

There are multiple markers of preeclampsia and are reported to be connected to the pathophysiology of the disease. Some are directly involved in the symptoms of disease while others are situated in the

Table 1. The expression, specific location in placenta and main function of various growth factors in normal pregnancy.

Name	Placental location	Expression	Function	References
VEGF	STB, iEVT	Rises in 3rd trimester	Induction of endothelial cell migration, division and survival.	30
PlGF	CTB, STB, EVT	Rises in 3rd trimester	Induce trophoblast cell invasion	31,32
TGF-β	STB, CTB	Rises in 1st trimester	Inhibit trophoblast cell proliferation and differentiation toward the invasive EVT pathway.	33,34
Activin A	CTB, EVT	Rises in 1st trimester	Stimulate hCG and progesterone secretion and CTB proliferation and increases EVT migration.	35–37
Inhibin A	STB	Expressed throughout pregnancy	Trophoblast cell endocrine and vascular development	37
Endoglin	STB, CTB	Rises in 1st trimester	Angiogenesis	38,39
Nodal	STB, EVT	Expressed throughout pregnancy	Inhibit proliferation and induce apoptosis in EVT cells	40,41

STB, syncytiotrophoblast; CTB, cytotrophoblast; EVT, extravillous trophoblast; iEVT, interstitial trophoblast

upstream of pathophysiological cascade. Studies have focussed on cytokines, indicators of endothelial dysfunction, markers of oxidative stress and angiogenic factors.[12,14,18,21–24]

In this review, we will only discuss studies on growth factors that are especially associated with early onset preeclampsia, which contributes to adverse maternal and perinatal outcomes.

Activin A and Inhibin A

Activin is one of the members of TGF-β superfamily. Activin A is a pleurotropic cytokine that plays a very important role in many developmental processes.[42,43] Mature Activin A is a dimer consisting of disulphide linked inhibin subunits. Activin A signals by binding and activating serine threonine kinase type II receptor (ActRIIA and ActRIIB).[35] Activation of type II receptor in turn recruits type I receptor (ALK_4) which then activates smad 2 and smad 3 proteins to induce target genes.[35,36] Activin A and its receptors are expressed in placental deciduas and uterine tissues during pregnancy.[44] *In vitro* studies have indicated that cell migration and invasion of trophoblast cells was induced by Activin A. Furthermore, Activin A also induces production of several hormones including GnRH, hCG, progesterone and matrix metalloproteinase (MMP2) by trophoblast cells. Studies have shown an increase in the maternal serum and placental inhibin A and Activin A levels in the pregnancies that subsequently developed preeclampsia.[37] Evidence has also suggested that Activin A is a promising biomarker for the detection of preeclampsia in Chinese populations. It is also reported that treatment of trophoblast cells with high doses of Activin A promotes apoptosis and therefore affects invasion of extravillous trophoblasts in the myometrium.[26]

Transforming growth factor β 1 and endoglin

Transforming growth factor β_1 (TGF β_1) is a multifunctional cytokine and is involved in several physiological processes including embryonic growth and development, repair-inflamation and angiogenesis.[33,45] It is reported that maternal symptoms of preeclampsia are produced by the release of TGF β_1 from endothelial cells in response to phagocytosis of necrotic trophoblasts in preeclamptic placentae. Therefore, TGF β_1 is involved in the pathogenesis of preeclampsia.[34] Endoglin is the co-receptor for TGF β_1 and TGF β_3 and modulates TGF β signalling by interacting with type I and type II receptors of TGF β (Table 2).[38] Endoglin is expressed by vascular endothelium and syncitiotrophoblasts. It is involved in the process of angiogenesis.[39] An increase in soluble endoglin is detected few weeks before the symptoms of preeclampsia; however, levels of TGF β_1 do not show any significant difference in normal vs. preeclamptic women.[38] There is an evidence of increase in the concentrations of endoglin with severity of the symptoms of preeclampsia.[18] Interestingly, elevation in the levels of endoglin is also reported in pregnancies with intrauterine growth restriction without maternal syndrome, suggesting that soluble endoglin is not a promising marker for preeclampsia.[46]

Table 2. Receptors involved in the downstream signal pathway of various growth factors.

Name of growth factor	Receptors
TGF β	Serine threonine kinase receptor (type I and type II)
Activin A	Serine theronine kinase receptor (ActRII A and ActRIIB = type II receptors and ALK_4 = type I receptor)
Nodal	Serine threonine kinase receptor (ALK_7 and ALK_4 = type I receptors)
VEGF A	Two tyrosine kinase receptor isoforms (VEGF-R1 = Flt1 and VEGF-R2)

ActRII, activin receptor type II; ALK, Activin receptor like kinase; VEGF-R, vascular endothelial growth factor receptor

Vascular endothelial growth factor A (VEGF-A)

VEGF-A produces many functions in endothelial cells including induction of angiogenesis, reduction of apoptosis and increase of vascular permeability.[47] It binds with high affinity to two tyrosine kinase receptors expressed on vascular endothelial cells (Table 2).[48] Hypoxia stimulates the expression of VEGF-A mRNA expression.[49] Fms-like tyrosine kinase receptor (FLT-I) is a receptor which binds with VEGF-A and placental growth factor (PIGF) with high affinity and is expressed in many human tissues, including placental trophoblasts. Its expression is also up-regulated by hypoxia conditions.[50] Literature review shows contradictory findings about the expression of VEGF family angiogenic growth factors in the placenta during pregnancy. The inconsistent results are due to the probes used in *in situ* hybridization studies and many cross react with other growth factors resulting in false-positive data.[51] Preeclampsia is associated with early placentation defects and inadequate maternal spiral artery remodelling. In preeclampsia, shallow endovascular invasion is the result of failure of cytotrophoblast differentiation into vascular phenotype. It is reported by *in vivo* and *in vitro* studies that preeclamptic placentae retain some adhesion molecules while fail to up-regulate others, which are normally expressed by most differentiated and invasive trophoblasts.[52,53] Studies have shown that cytotrophoblasts are regulated by VEGF and blocking of VEGF ligand significantly decreases the expression of integrin α1 and induces apoptosis in these cells.[54] Increasing evidence has suggested that a decrease in the expression of VEGF-A and FLT-1 is noticed in severe preeclampsia, as compared to normal placentae.[51] Recent studies have also reported that shallow invasion of extravillous trophoblast and impaired spiral artery remodelling not only lead to defective utero-placental circulation but also causes damage to chorionic villi, leading to clinical features of pre-eclampsia.[55] There is consistent evidence of increase in maternal serum and placental expression of FLT-1 in preeclamptic women as compared to normal pregnant women.[47,56] Moreover, levels of FLT-1 are directly proportional to the degree of proteinuria.[56] Although, VEGF-A plays a promising role in normal pregnancy and in the pathogenesis of preeclampsia, it has a limited clinical role in the prediction of preeclampsia due to extremely low circulating levels of free VEGF-A, below the detection level of ELISA kits. The potential use of VEGF family in the treatment of preeclampsia is explored by using many animal models. Infection of pregnant rats with overexpressed FLT adenovirus resulted in hypertension and proteinuria, with renal lesions associated with preeclampsia in pregnant women. Reduction in hypertention and proteinuria and improvement in glomerular endotheliosis was also noticed by induction of recombinant VEGF-A in these rats.[57] Recently, potential use of the VEGF family in the treatment of preeclampsia is being explored and many findings have suggested that VEGF-A may have a therapeutic potential in the management of preeclampsia. However further studies on the possibility of potential adverse effects of VEGF-A therapy will be beneficial to completely understand the value of VEGF-A in early diagnosis and treatment of preeclampsia.[51]

Placental growth factor (PIGF)

There is 42% amino acid sequence identity between VEGF-A and PIGF.[58] PIGF promotes angiogenesis and the level of maternal serum PIGF is inversely proportional to FLT-1 levels. In preeclampsia, levels of PIGF are lower as compared to normal pregnancy, with the most pronounced difference observed 5 weeks before the clinical onset of the disease.[31,32,59,60] PIGF is a small molecule (30KD), freely filtered by glomerulus and can be found in urine. It can easily be detected by dipstick technology.[61] It is proposed in many studies that the ratio between PIFF and FLT-1 in early-mid pregnancy is a screening tool for preeclampsia that led to these biomarkers being validated for routine clinical use in some countries. However, highest predictive values have not been achieved.[51] One scenario is to screen all women with low PIGF urine concentration and to consider all those with low levels as high-risk women.

Serial serum measurements of FLT-1 and PlGF could then be performed to identify more precisely women at high risk.[31] However, studies have suggested that prediction of preeclampsia by comparing ratios of FLT-1 to PlGF have highest predictive values during second trimester screening.[51] A screening test has been launched by Roche in Europe, as a diagnostic screening procedure for preeclampsia in the second trimester.[18] This test can significantly separate healthy women and women with preeclampsia when screening was performed after 20 weeks of gestation.[62] However, sensitivity and accuracy of test need to be improved to decrease the number of false positive and false negative patients. This can prevent over-diagnosis/over-treatment, reduce cost of monitoring by reducing unnecessary hospital admissions and improve earlier detection and appropriate management.[1]

Nodal

Nodal is a member of transforming growth factor beta superfamily. Nodal signals through type II and type I serine/threonine kinase transmembrane receptor proteins. Nodal has been reported to act through two type I receptors (activin receptor-like kinase 4 and 7) (ALK4 and ALK7) (Table 2).[40,63] A preeclampsia susceptibility locus is located on chromosome 2q22 along with type II receptor and ALK7, suggesting an association of these receptors with preeclampsia.[64,65] Several studies have suggested that Nodal is important for placental development. Abnormal placentation is reported due to a mutation of the Nodal gene, which leads to expansion of giant cells and spongiotrophoblast layers and decreases the labyrinthine development.[66] Nodal also inhibit precocious differentiation of trophoblast stem cells by acting on extraembryonic stem cells.[67] In human placenta, Nodal and its receptor ALK7 has been expressed. It is also reported that Nodal signals through ALK7, inhibits trophoblast proliferation and induces apoptosis.[40,41] Recently spatial and temporal expression patterns of Nodal and ALK7 in human placenta were examined and level of expression of Nodal and ALK7 were found to be up-regulated in severe early-onset preclamptic placenta. Furthermore, Nodal and ALK7 signals were also detected in the villous mesenchyme surrounding the paravascular capillary network of the intermediate villi, suggesting a potential role of Nodal in angiogenesis or vascular control. It is also reported that overexpression of Nodal and ALK7 significantly decreases trophoblast cell migration and invasion. Most promising data came from a study on human placental explants. Placental explants cultures showed expansion of explants and migration of extravillous trophoblast cells, when treated with Nodal small-interferring RNA. Invasion of EVT cells also depend on the degradation of extracellular matrix by MMP-2 and MMP-9, and their activity is controlled by Tissue inhibitor of matrix metalloproteinases 1(TIMP 1). It was also determined that Nodal inhibits trophoblast cell invasion and migration partly by acting through TIMP1-MMP-2/MMP-9 pathway. Increased expression of Nodal/ALk7 in extravillous trophoblast cells may result in defective cytotrophoblast differentiation, shallow invasion of uterus, excessive apoptosis and imbalance of MMP2/TIMP1 ratio.[68] It is also reported that increased level of Activin A in preeclamptic placenta enhances Nodal signalling,which induces apoptosis of trophoblast cells.[26] All these findings suggest that Nodal/ALK7 pathway is involved in the regulation of placental development and function and that defective signalling of Nodal may contribute to the pathogenesis of preeclampsia.

DISCUSSION

Preeclampsia is a serious and complicated disease. Although its causes are not entirely clear, shallow trophoblast invasion and excessive apoptosis along with dysfunction in spiral artery remodelling leads to preeclampsia.[1,11,12,18,25,68] Many of the studies summarized here support the idea that various protein biomarkers are known and their level of expressions and roles in pregnancy complications have studied, mainly in preeclampsia.[12] However, researchers found no widely acceptable and promising marker for the early detection and prevention/treatment of disease. There is an increasing need of an affordable and widely applicable test that could permit early detection of preeclampsia to identify and monitor high risk pregnant mothers and to provide the best prenatal care for them and for their child.

Extensive preclinical and clinical studies have demonstrated that FLT 1, VEGF-A, PlGF and their ratios play important roles and may be useful markers in prediction of preeclampsia.[11,12,18,69] The biggest challenge is limitations in their clinical utility, since high precision tests can only be performed in second trimester of gestation. There is an increasing interest in combining several variables, as no single test can predict preeclampsia with accuracy.

Recent studies on Nodal and its role in placental development have suggested the need of future studies to focus on the expression pattern and function of Nodal in the placentae of other populations.

It will provide interesting insights into the clear and well defined role of Nodal and its signalling in preeclampsia.

Identification of women at risk for preeclampsia is important not only to develop and evaluate preventive treatments but also to improve the structure of antenatal care. Further studies need to be carried out to find affordable and precise methods of early detection of preeclampsia. These studies are also needed to suggest therapeutic potential in the management of preeclampsia.

COMPETING INTERESTS

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