

# The Utility of Vascular Dysfunction Studies in the Prediction and Prevention of Preeclampsia: A Historical Review

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**Abstract:** Preeclampsia is a major cause of maternal and perinatal mortality. Although preeclampsia may be caused by several factors, endothelial cell dysfunction has been proposed as the main pathophysiological cause. Dysfunctional endothelium in the uteroplacental circulation not only increases peripheral vascular resistance, but also affects generalized vasoconstriction *via* humoral factors released from the placenta. A standard method for predicting and preventing preeclampsia has yet to be developed; however, the analysis of a combination of biochemical markers, particularly markers related to vascular dysfunction, may enhance our ability to predict and prevent preeclampsia in the near future.

**Keywords:** Angiogenic growth factor, endothelial cell activation, inflammatory response, oxidative stress, preeclampsia.

## INTRODUCTION

Preeclampsia, known as gestational hypertension with proteinuria, complicates 5% to 8% of all pregnancies. Despite the current state of perinatal care, preeclampsia continues to be associated with high perinatal mortality and intrauterine fetal growth retardation [1]. In cases of severe preeclampsia after the 28th gestational week, early delivery is typically preferred in order to improve the neonatal prognosis. Ninety percent of preeclampsia occurs after 34 weeks' gestation, the time at which prognosis is relatively good. Early-onset preeclampsia (before 32 weeks gestation), however, can be serious; and delivery can be associated with elevated neonatal morbidity and mortality rates in preeclamptic women [2, 3]. At-risk pregnant women who develop preeclampsia should be subjected to close maternal and fetal surveillance, especially in the second and early third trimesters [2]. Low-calorie and appropriate sodium-restricted diets may retard the development of preeclampsia and thereby improve perinatal outcome [3].

The development of methods to predict and prevent preeclampsia in early pregnancy is important for management of this condition. Although many investigators have sought to develop practical screening tests for the prediction of preeclampsia, these tests have not attained sufficient sensitivity and specificity [4]. Recent research on vascular dysfunction, however, has provided results that may lead to the successful and reliable prediction of preeclampsia.

In this study, we reviewed the history of approaches employed to predict preeclampsia and prevent its onset. The first section includes a discussion of traditional prediction methods, of which uterine arterial doppler measurement dur-

ing the midtrimester has been the most effective. The second section presents the use of biochemical markers as a modern approach to preeclampsia prediction. Vascular dysfunction is a major cause of preeclampsia, and biochemical markers related to vascular function have been reported to predict preeclampsia onset. The third section reviews recent trials of treatments intended to prevent the onset of preeclampsia, including the use of vitamin C and E. This field of study shows promising progress for preeclampsia prevention in the near future.

## RISK FACTORS FOR PREECLAMPSIA

Women experiencing high-risk pregnancies should be identified in order to predict and prevent preeclampsia occurrence [1, 4-7]. Although mild preeclampsia shows no familial tendency, family history of severe preeclampsia is a risk factor. In a study cohort of preeclamptic women, the mother of the preeclamptic women had a 14% rate of a history of severe preeclampsia, while unrelated controls (their mothers-in-law) had a rate of only 3% [8]. Nulliparity is another risk factor, as preeclampsia has been diagnosed among 64% of nulliparous women and only 36% of parous women [9]. Women with a body mass index greater than 35 before pregnancy have a four-fold greater rate of preeclampsia than those with a body mass index in the range of 19-27 [10]. Furthermore, a low body mass index (<20) reduces the prevalence of preeclampsia [11]. Multiple gestation is known to be a strong risk factor for preeclampsia. Coonrod *et al.* [12] reported that twin pregnancy carried a nearly four-fold increased risk for preeclampsia. Furthermore, pregnant women aged 40 years and older were more likely to develop preeclampsia (nulliparous: OR 1.8, 95% CI 1.3-2.6; multiparous: OR 1.9, 95% CI 1.2-2.9) leading to increased maternal morbidity [13].

Complications also contribute to the risk for preeclampsia. The incidence of preeclampsia was reportedly increased

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to 9.9% in diabetic pregnancies compared with 4.3% in non-diabetic controls [14]. Untreated hyperthyroidism is also known to stimulate preeclampsia-eclampsia; however, diagnosis of hyperthyroidism can reduce the incidence of preeclampsia through prompt management [15]. The prevalence of preeclampsia (11.7%) in anti-cardiolipin antibody-positive women is significantly higher than that in other pregnant women (OR 1.9, 95% CI 2.43-16.0) [16]. On the other hand, it has been reported that long-term semen exposure [17] and T cell immune deficiency [18] reduce the risk of preeclampsia.

## CLASSICAL PREDICTIVE APPROACHES

Many methods intended to predict the onset of preeclampsia have been investigated throughout the history of research on this disease. These approaches have been based on the theory that vascular reactivity is elevated in pregnant women who develop preeclampsia. Although vascular responsiveness to angiotensin II (AngII) decreases early in normal pregnancy, pregnant women who subsequently develop preeclampsia are extremely sensitive to the pressor effects of infused AngII [19].

### Blood Pressure

In normal pregnancy, blood pressure is lowest between 16 and 20 weeks' gestation and then gradually increases toward non-pregnant levels in the late stages of pregnancy. The mean level of diastolic blood pressure is usually less than 75 mmHg in the middle of a normal pregnancy. Since vascular reactivity (especially to AngII) is stimulated in preeclampsia, the elevation of diastolic blood pressure may be a good predictor of preeclampsia. Caritis *et al.* [5] reported that the risk of preeclampsia was 8% for women with a mean arterial pressure of <75 mmHg vs. 27% for women with a mean arterial pressure >85 mmHg. However, the predictive power for preeclampsia is very low (20%) when the cutoff level for diastolic blood pressure is set at 80-85 mmHg [20]. Simple blood pressure tests are thus not effective in predicting the occurrence of preeclampsia.

### Roll Over Test (ROT)

The ROT evaluates the risk of preeclampsia by measuring elevation of blood pressure stimulated by a change of maternal position [21]. In the ROT, diastolic blood pressure levels during bed rest and after the change of maternal position are measured, and the difference between the two values is calculated. A positive ROT is defined as an elevation in diastolic blood pressure greater than 20 mmHg. The negative predictive value of this test is relatively high and its reproducibility is poor [22, 23].

### Handgrip Test (HGT)

The HGT, performed before the 15th gestational week, measures the change in blood pressure induced by 3-5 min of sustained isometric exercise. Test results are considered positive if systolic blood pressure increases by 15 mmHg or more during the isometric exercise or decreases by 14 mmHg or more immediately after exercising. The negative predictive value of this test is high (98%) [24].

## Angiotensin Sensitivity Test (AST)

Although vascular sensitivity to AngII is reduced in normal pregnancy, pregnant women who develop preeclampsia show increased sensitivity to AngII [25]. The AST measures the dose of AngII required to raise systolic blood pressure by 20 mmHg (effective pressor dose) at 26-32 weeks gestation. It has been reported that an effective pressor dose less than 12 ng/kg/min can yield a high percentage of false-positive results [25]. This method is complicated and invasive, and the positive and negative predictive values are poor. Therefore, AST is not considered to be an effective screening test for preeclampsia [26].

## Doppler Ultrasonography (uterine arterial blood flow)

In preeclampsia, trophoblast invasion into the uterine myometrium is poor and the reconstruction of spiral arteries fails. As a result, utero-placental perfusion is reduced. Reduced utero-placental blood flow can be measured with ultrasonography since uterine arterial flow reflects the change of spiral arterial flow [27]. Reduction in blood flow may be estimated from an elevation in the uterine arterial pulsatility index (PI) [28] or resistance index (RI) [29]. Also, the uterine arterial notch may be associated with the ischemic pathology of a preeclamptic placenta [30]. However, uterine arterial doppler measurement in mid trimester has been found to be deficient as a screening test for preeclampsia [31]. The reliability of these indices (PI, RI) is low since they are affected by multiple factors including placental position, uterine contractions, and maternal heartbeat. However, the examination has merits as an easy and non-invasive method. The sensitivity is 75%, but the negative predictive value is high (88%) [32].

## MODERN PREDICTIVE APPROACHES

### Endothelial Cell Activation or Dysfunction

While the pathophysiology of preeclampsia remains unclear, activation or dysfunction of the vascular endothelium in uteroplacental circulation has been proposed as a possible cause of preeclampsia [33]. Dysfunctional endothelium in the uteroplacental circulation not only increases peripheral vascular resistance, but also influences generalized vasoconstriction *via* humoral factors released from the placenta. Therefore, hypertension rapidly improves after delivery [33]. Since endothelial cells maintain the homeostasis of the coagulation cascade [34], the inflammatory process [35], and vascular tone [36], endothelial cell activation or dysfunction can affect thrombosis [34], inflammation [35], and hypertension [36].

In preeclampsia, increased vascular permeability decreases circulating plasma volume, which results in hemoconcentration. The vascular endothelial dysfunction that accompanies hemoconcentration can then lead to hypertensive disorder [37]. Placental ischemia, which is a characteristic of preeclampsia, is associated with a release of molecules from endothelial cells. Fibronectin, a high-molecular weight extracellular matrix glycoprotein produced by the endothelium, is involved in cellular interactions such as cell adhesion and migration. Plasma fibronectin concentration, which is thought to be an indicator of endothelial injury, is signifi-

cantly increased in women with preeclampsia [38, 39], even before its clinical onset [40]. As mentioned above, endothelial cellular abnormality in the uteroplacental and fetoplacental circulation has recently attracted widespread attention as a possible cause of preeclampsia [33].

### Coagulation Cascade

Platelet aggregation and coagulation cascade are stimulated in preeclampsia. Emonts *et al.* [41] attempted to develop a prediction index using APTT, PT, activated factor VIII, and free protein S in combination with relative plasma volume and maternal history of chronic hypertension. They demonstrated a satisfactory positive predictive value and cost efficiency ratio. Chappell *et al.* [42] demonstrated that an algorithm analysis involving the plasminogen activator inhibitor-1/plasminogen activator inhibitor-2 ratio and placenta growth factor (PlGF) could effectively predict preeclampsia.

### Inflammatory Response

Endothelial activation or dysfunction initiated by lipid peroxidation and leukocyte activation may be a key process in the pathogenesis of preeclampsia. This possibility has been suggested because many indicators of a systemic inflammatory response are markedly changed in preeclampsia [43] and large numbers of inflammatory cells permeate outside the placental blood vessels during preeclampsia [44]. Rajmakers *et al.* reported that infusion of TNF $\alpha$  and a pro-inflammatory cytokine caused hypertension in pregnant rats [45]. Indeed, serum concentration of TNF $\alpha$  has been reported to be increased even before the onset of preeclampsia [46]. Furthermore, pregnant women who develop preeclampsia also have a high serum concentration of the soluble adhesion molecules sE-selectin and sICAM-1 in the first trimester [46]. TNF $\alpha$  promotes ICAM-1 and E-selectin activation on endothelial cells [47] and facilitates the membrane-bound ICAM-1 and E-selectin into the blood by shedding [48]. We suggest that the excessive amount of TNF $\alpha$  produced in the placenta early in pregnancy activates endothelial cells and promotes the expression of adhesion molecules. Endothelial activation or dysfunction caused by inflammatory reaction could reduce the production of prostacyclin [49] and nitric oxide [50], leading to impaired vascular relaxation. Markers of pro-inflammation and inflammation such as serum TNF $\alpha$ , sE-selectin, and sICAM-1 might be predictive of preeclampsia.

### Angiogenic Growth Factor

The pathophysiology of preeclampsia begins with placental dysfunction in the first trimester [51]. In normal pregnancy, extravillous trophoblasts travel to the maternal spiral arteries during the first trimester [52]. Vascular endothelial growth factor (VEGF) and PlGF, which are strong proangiogenic proteins, are expressed in the trophoblasts and play a role in the process of spiral artery remodeling in normal pregnancy [53, 54]. Adequate placentation relies on appropriate fetoplacental vascular networking including spiral artery remodeling. VEGF stimulates endothelial cell growth and migration, and promotes angiogenesis during embryonic development. PlGF shares 53% homology with VEGF at the amino acid level and its angiogenic function is similar to

VEGF. Trophoblasts strongly express PlGF and the receptor is predominantly expressed. Serum PlGF levels rise during the first and second trimesters of pregnancy, peaking early in the third trimester [55]. They are involved in an early stage of the process of placentation. In preeclampsia, free PlGF concentration in serum was found to be reduced compared with normal pregnancy [55, 56], and the serum level was already reduced before 20 weeks' gestation in women who would later develop preeclampsia [55]. Decreased levels of free PlGF could influence endothelial cell and trophoblast function, thereby contributing to the pathogenesis of preeclampsia.

Soluble fms-like tyrosine kinase 1 (sFlt-1), a circulating anti-angiogenic protein, is increased in the placenta and serum of women with preeclampsia [57]. sFlt-1 binds with VEGF and PlGF, preventing their interaction with endothelial Flt-1 and KDR and thereby inducing endothelial dysfunction. Increased circulating sFlt-1 concentration beginning five weeks before the onset of preeclampsia is accompanied by decreases in free PlGF and VEGF levels in maternal circulation. Measurement of the serial concentrations of sFlt-1 and free PlGF throughout pregnancy may allow researchers to better assess the relevance of these markers in the early identification of preeclampsia [58].

### Cell-Free Fetal DNA in Maternal Circulation

Cell-free fetal DNA in maternal circulation allows non-invasive prenatal diagnosis [59] since cell-free DNA can be derived from fetal white blood cells or fetal nucleated red blood cells. Fetal DNA in maternal circulation, however, is primarily derived from the placenta [60] since cell-free DNA is not correlated with fetal white or nucleated red blood cell counts, or the amount of fetal DNA [61].

Recent analysis of placental DNA and RNA in maternal plasma indicates that the nucleotide molecules could represent a preeclampsia predicting marker. There is a significant increase in the shedding of cellular debris, including syncytiotrophoblast microparticles, in preeclampsia that is not seen in normal pregnancy. It is thought that the debris results from normal renewal and repair, and that it is increased in preeclampsia due to syncytial apoptosis caused by placental hypoxia [62]. Syncytiotrophoblast microparticles, including cell-free DNA and mRNA, are released from the surface of the placenta into the maternal circulation. Mechanical damage to placental syncytiotrophoblasts may particularly increase syncytiotrophoblast microparticles in maternal circulation during preeclampsia [63]. A recent study reports that placenta-derived hypermethylated *RASSF1A* gene, a tumor suppressor gene, in maternal circulation can be utilized as a universal fetal marker regardless of fetal sex or genetic variations [64]. Furthermore, Tsui *et al.* insist that quantitative aberrations of hypermethylated *RASSF1A* in the maternal plasma of preeclamptic women represent a potentially useful non-invasive measure for the prenatal assessment of preeclampsia [65].

### Neurokinin B

The biological actions of neurokinin B, a tachykinin peptide in the family of peptides that comprise substance P, include smooth muscle contraction, pain transmission, neuro-

genic inflammation, and activation of the immune system [66A]. However, neurokinin B is undetectable in the peripheral tissues of non-pregnant animals [67A]. Neurokinin B is expressed at the outer syncytiotrophoblast of the placenta, and its secretion into both fetal and maternal circulation originates mainly from the placenta. Normally, neurokinin B can be detected in plasma as early as the ninth week of gestation, increases in mid and late pregnancy, and then decreases rapidly after delivery [68]. Plasma concentration of neurokinin B is markedly elevated in preeclampsia [67, 69, 70]. Expression of the neurokinin B receptor TAC3 is also significantly higher in preeclamptic placenta [71]. During pregnancy, activation of TAC3 by neurokinin B reduces the blood flow through the liver to satisfy the needs of the uterus and placenta.

### Oxidative Stress

Hyperuricemia is a common finding in preeclampsia. It is usually considered secondary to altered renal function, increased tissue breakdown, and increased oxidative stress [72]. Uric acid is converted from the major purine nucleosides adenosine and guanosine through the purine catabolic pathway. The beneficial effects of uric acid include its antioxidant activity [73] and reduced nitrosylation of tyrosine residues on proteins by peroxynitrite [74]. Conversely, uric acid has pro-oxidant activity when other antioxidants are at low levels [75], and stimulates the inflammatory process that leads to endothelial dysfunction [76]. Uric acid-induced endothelial dysfunction could promote hypertension, vascular disease, and renal disease [76].

In normal pregnancy, uric acid has an antioxidant function [77]. The urate free radical created by the oxidation of uric acid [78], however, has the potential to go on to oxidatively modify placental proteins and lipids [79], leading to disturbed placentation and placental function. In women who develop preeclampsia, the plasma concentration of uric acid is increased at around 10 weeks of gestation—a development that precedes the clinical presentation of preeclampsia [80]. An increased serum uric acid level indicates underlying oxidative stress as these increased levels correlate with plasma hydrogen peroxide and plasma protein carbonyl levels in preeclampsia [81]. Increased placental oxidative stress in preeclampsia results from increased pro-oxidant generation coupled with insufficient antioxidant capacity. Furthermore, the plasma level of malondialdehyde, a major metabolite of lipid peroxide is increased in patients with preeclampsia [82]. Release of lipid peroxides into the maternal circulation and cytokine synthesis from activated neutrophils could contribute to maternal endothelial cell activation in preeclampsia [83].

Nicotinamide adenine dinucleotide phosphate-oxidase (NAD(P)H oxidase) in the placenta could be an important source of superoxide during pregnancy and preeclampsia [45]. On the other hand, antioxidant capacity is known to be decreased in preeclampsia [84]. Decreased plasma levels of the major intracellular antioxidant glutathione [85] and vitamin C [86] have been reported in women with preeclampsia. Hence, converting evidence suggests the pathophysiology of preeclampsia is characterized by increased oxidative stress and chronic inflammation.

### Neural Role in Vascular Dysfunction

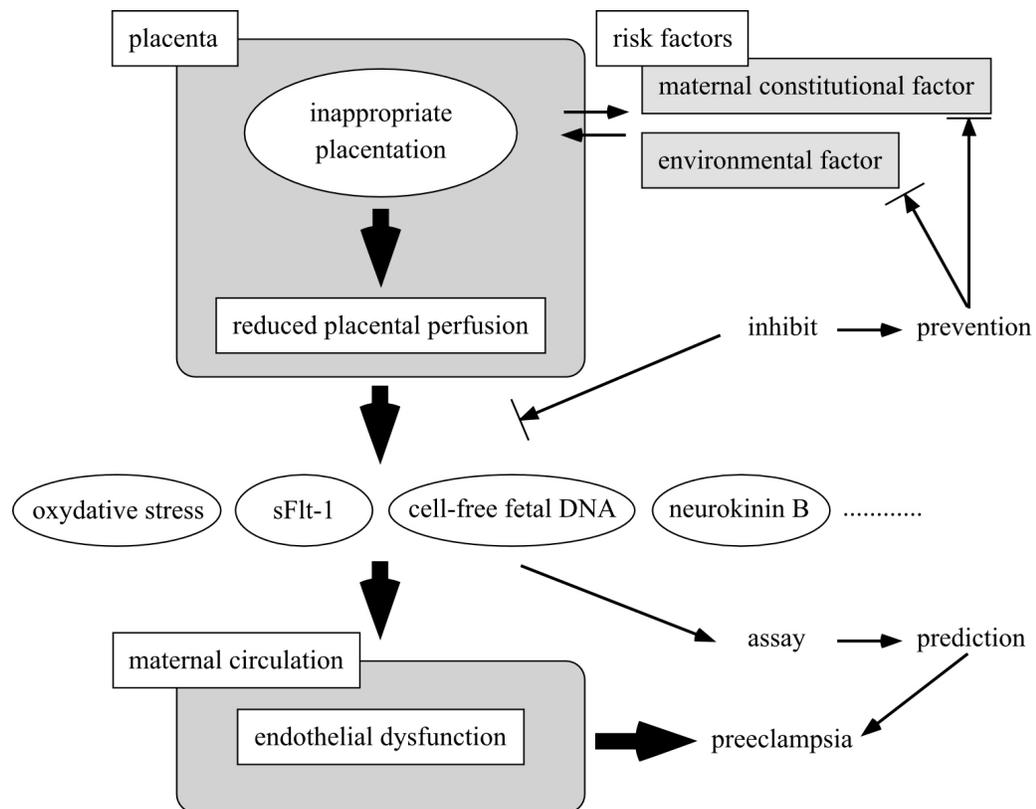
Quinn and colleagues suggested that a loss of vascular regulation in the uteroplacental circulation could result in uterine vasoconstriction, renal vasoconstriction, and maternal hypertension. This explanation is possible given that partial denervation at the endometrial–myometrial interface would prevent appropriate trophoblast invasion with a failure of adaptation of the uterine spiral arteries and generate a maternal cytokine response [87]. Given that regional uterine denervation related to curettage and back injury has been associated with cytokine responses including VEGF [88], investigation of some kinds of cytokines including sFlt-1 and neural originated cytokines, may be useful in elucidating a method for predicting onset of preeclampsia.

### PREVENTION OF PREECLAMPSIA

Low-sodium diets, calcium supplementation [89], and bed rest have been used in traditional preventive management for preeclampsia. A large trial suggested that low-dose aspirin could reduce the occurrence of preeclampsia among high-risk women [90-92]. Conversely however, Caritis *et al.* [93] reported that low-dose aspirin treatment did not reduce the incidence of preeclampsia or improve perinatal outcomes in high-risk pregnant women.

Reduced intake of calcium is known to be associated with an elevated risk of preeclampsia [94]. Meanwhile pregnant women with high calcium intake levels had a very low incidence of eclampsia despite the presence of high-risk factors [95]. Biological demand for calcium is very high during pregnancy and calcium is necessary for fetal growth. Thus, it is not surprising that calcium supplementation was reported to help prevent preeclampsia among high-risk patients [94]. Other investigators, however, have not found significant effects related to high calcium intake [1, 96]. It is possible that calcium supplementation may help preventing preeclampsia in populations whose typical diet is low in calcium [96A].

Diets rich in fruits and vegetables are associated with decreased mortality from cardiovascular disease [97]. A 37% reduction of fruit intake is also associated with preeclampsia risk [98]. Furthermore, a diet rich in vegetables may alleviate the signs and symptoms of preeclampsia [99]. This association suggests that vitamins C and E afford protection against cardiovascular disease and preeclampsia by reducing oxidative tissue damage [100]. Vitamin E can interfere with NAD(P)H oxidase activity through the inhibition of protein kinase C, leading to reduction of free radical generation [101]. Daily vitamin E supplementation in the dose range of 100-400 IU over 2 years or more was reported to be most efficacious in reducing low-density lipoprotein oxidation and positively influencing mortality rates from cardiovascular disease [100]. This antioxidant activity may also be beneficial for the prevention of preeclampsia due to its anti-inflammatory effect. Persons with diabetes or hypertension have also been reported to benefit from supplemental vitamin C intake [100]. Chappell *et al.* reported that a combination of vitamins C and E could reduce the incidence of preeclampsia among high-risk women [102]. However, randomized trials failed to show any consistent benefit if vitamins C and E on cardiovascular disease [103, 104].



**Fig. (1).** Reduced placental perfusion is a key of the pathophysiology of preeclampsia. Soluble mediators from preeclamptic placenta lead to maternal systemic vascular endothelial dysfunction. The analysis of soluble mediators could develop the way to predict and prevent preeclampsia.

**PERSPECTIVE**

Preeclampsia is a complex disease with multifactorial causes. To date, investigations of its prediction and prevention have not been completely successful. Clinicians do not yet have predictive and preventive standards for preeclampsia. Development of an effective combination of biochemical markers is necessary to identify high-risk women and to take steps to prevent preeclampsia occurrence (Fig. 1). Recently, DNA and RNA [105] in plasma derived from preeclamptic patients has been investigated to predict the occurrence of preeclampsia. Multivariable analysis of a combination of DNA, RNA, and protein in plasma might be needed in order to achieve a reliable method for predicting preeclampsia onset. We hope that recent findings in the field of blood analysis may further our abilities to manage preeclampsia in the future. The prediction and prevention of preeclampsia remains a clinical issue in maternal and fetal health.

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