

Is inflammation the cause of pre-eclampsia?

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Abstract

It has been proposed that either excessive inflammation or an imbalance in angiogenic factors cause pre-eclampsia. In the present review, the arguments for and against the role of inflammation and/or angiogenic imbalance as the cause of pre-eclampsia are discussed on the basis of the Bradford-Hill criteria for disease causation. Although both angiogenic imbalance and systemic inflammation are implicated in pre-eclampsia, the absence of temporality of inflammatory markers with pre-eclampsia challenges the concept that excessive inflammation is the cause of pre-eclampsia. In contrast, the elevation of anti-angiogenic factors that precede the clinical signs of pre-eclampsia fulfils the criterion of temporality. The second most important criterion is the dose-response relationship. Although such a relationship has not been proven between pro-inflammatory cytokines and pre-eclampsia, high levels of anti-angiogenic factors have been shown to correlate with increased incidence and disease severity, hence satisfying this condition. Finally, as the removal of circulating sFlt-1 (soluble Fms-like tyrosine kinase receptor-1) from pre-eclamptic patients significantly improves the clinical outcome, it fulfils the Hill's experiment principle, which states that removal of the cause by an appropriate experimental regimen should ameliorate the condition. In contrast, treatment with high doses of corticosteroid fails to improve maternal outcome in pre-eclampsia, despite suppressing inflammation. Inflammation may enhance the pathology induced by the imbalance in the angiogenic factors, but does not by itself cause pre-eclampsia. Development of therapies based on the angiogenic and cytoprotective mechanisms seems more promising.

Background

Pre-eclampsia is an important cause of maternal and perinatal mortality affecting 5–7% of pregnant women [1]. Clinically, it is defined as the *de novo* onset of hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg) and proteinuria (≥ 300 mg/24 h) after 20 weeks of gestation. In extreme cases, serious complications of pre-eclampsia can include acute renal failure, seizures (eclampsia), pulmonary oedema, acute liver injury, haemolysis and/or thrombocytopenia. The last three signs occur together as part of the HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome, a severe variant of pre-eclampsia. Apart from the hypertension and proteinuria, the central nervous system also plays a role, such as headache and hyperreflexia.

Pre-eclampsia has been cited as “the disease of theories” because of its unknown aetiology [2]. In recent years, endothelial dysfunction has emerged as the leading phenomenon responsible for the clinical signs of the disorder [3]. Endothelial dysfunction is commonly linked

to the impairment of the NO (nitric oxide) pathway and decrease in NO bioavailability [4,5]. Factors proposed to cause endothelial dysfunction in pre-eclampsia include poor placental vascular remodelling and placental ischaemia, oxidative stress [6,7], excessive inflammation [8], imbalance in angiogenic factors [9–12] and the loss of endogenous protective regulators [3,13–15].

Poor placentation and reduction in uterine blood flow (placental ischaemia) has for a long time been proposed to be the leading cause for the increase in oxidative and endoplasmic reticulum stress, production of potent pro-inflammatory mediators and anti-angiogenic factors in pre-eclampsia [16,17]. However, the role of inadequate placental vascular remodelling due to defective trophoblast invasion as the leading cause of pre-eclampsia has been disputed on the basis of evidence that failure to remodel the uterine arteries is also associated with intrauterine growth restriction, where no signs of hypertension and proteinuria are observed [18].

More recently, the ‘excessive inflammation’ and the ‘angiogenic imbalance’ theories were highlighted as the cause of pre-eclampsia. Although a generalized systemic inflammation is common to all pregnancies [19], Redman et al. [8] proposed that pre-eclampsia is not intrinsically different from normal pregnancy, but it is at the extreme end of a continuous spectrum of inflammatory responses that are a feature of pregnancy itself. In parallel, Ahmed [9] had proposed that

Key words: cytokine, inflammation, pre-eclampsia, soluble endoglin (sEng), soluble Fms-like tyrosine kinase receptor-1 (sFlt-1), vascular endothelial growth factor (VEGF).

Abbreviations used: eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; HELLP, haemolysis, elevated liver enzymes and low platelets; IFN γ , interferon γ ; IL, interleukin; PlGF, placenta growth factor; sEng, soluble endoglin; sFlt-1, soluble Fms-like tyrosine kinase receptor-1; TNF α , tumour necrosis factor α ; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

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Table 1 | Bradford-Hill criteria of causation

Criterion	Explanation
Temporality	Exposure always precedes the outcome
Dose-response	Increasing amount of exposure increases the risk
Experiment	The condition can be altered (prevented or ameliorated) by an appropriate experimental regimen
Strength	The stronger the association, the more likely it is that the relationship of 'A' to 'B' is causal
Consistency	The association is consistent when results are replicated in studies in different settings using different methods
Specificity	When a single putative cause produces a specific effect
Coherence	The association should be compatible with existing theory and knowledge
Plausibility	There needs to be some theoretical basis for positing an association between a vector and disease

it is the imbalance in angiogenic factors that cause pre-eclampsia; in particular, the increase in sFlt-1 (soluble Fms-like tyrosine kinase receptor-1) and sEng (soluble endoglin) and the decrease in PlGF (placenta growth factor). In the present review, the arguments for the role of inflammation and angiogenic imbalance as the cause of pre-eclampsia are discussed on the basis of the Bradford-Hill criteria for disease causation (Table 1).

Evidence of the role of angiogenic factors in pre-eclampsia

In 1997, the angiogenic imbalance hypothesis proposed that pre-eclampsia arises due to loss of VEGF (vascular endothelial growth factor) activity as a result of rise in sFlt-1 [9]. This gained support when it was shown that sFlt-1 levels increased in the amniotic fluid [20] and placenta [21] of pre-eclamptic women. The role of sFlt-1 in the aetiology of pre-eclampsia acquired serious recognition when it was shown that adenoviral overexpression of sFlt-1 in pregnant rats mimicked the clinical manifestations of pre-eclampsia [10]. Indeed, anti-VEGF therapy also promotes hypertension and proteinuria in cancer patients [22]. Furthermore, the circulating level of maternal sFlt-1 is significantly elevated before the onset of the disorder and persisted until delivery [23]. High levels of sFlt-1 causes endothelial dysfunction [10,24,25] and inhibits angiogenesis [26,27] by binding to and antagonizing the function of both VEGF and PlGF [28]. In addition, the circulating levels of VEGF and PlGF are decreased before the onset of pre-eclampsia [12,29]. Both VEGF [30,31] and PlGF [32] have been shown to stimulate NO release [33], indicating that their loss would lead to a reduction in NO production. Like sFlt-1, sEng is elevated early in pre-eclampsia [11] and limits the activity of TGF β 1 (transforming growth factor β 1) signalling and eNOS

(endothelial nitric oxide synthase) [34,35], hence promoting vascular dysfunction and inhibiting angiogenesis [11].

Evidence of inflammation in pre-eclampsia

Both observational and experimental studies have demonstrated an association between inflammation and endothelial dysfunction [36,37]. Redman et al. [8] first proposed that pre-eclampsia arises as a result of an excessive maternal intravascular inflammatory response to pregnancy, which may occur because either the stimulus or the maternal response is too strong and involves both the innate and the adaptive immune system. Two decades ago, Greer et al. [38] first showed that neutrophil activation is confined to the maternal circulation in pregnancy-induced hypertension, where it may contribute to vascular damage. Soluble markers of neutrophil activation, released in the circulation from the degranulation of activated neutrophils, are also increased in pre-eclamptic patients. [39–42]. Further evidence of enhanced inflammation in pre-eclampsia has been demonstrated through the uncontrolled increased activation of the complement system compared with normal pregnancy. Activation of the complement system amplifies inflammation, promotes chemotaxis of inflammatory cells and generates proteolytic fragments that enhance phagocytosis by neutrophils and monocytes [43].

Another important feature of systemic inflammation in pre-eclampsia is the predominance of Th1-type immunity and the absence of Th2 tendency. T-cells may be classified as Th1 cells, which synthesize IL (interleukin)-2, IL-12, IFN γ (interferon γ) and TNF α (tumour necrosis factor α), and induce cellular immunity, or Th2 cells, which synthesize IL-4, IL-5, IL-6 and IL-10, and induce antibody production [44]. Normal pregnancy is characterized by a shift towards Th2-type immunity and the inhibition of cytotoxic Th1 immune responses, which could be harmful to the fetus [45]. In pre-eclamptic patients, the decidual lymphocytes and peripheral blood mononuclear cells are generally primed to synthesize high levels of Th1 cytokines. In contrast, the same patients exhibited low spontaneous or phytohaemagglutinin-induced expression of the Th2 cytokines [46–48]. In addition, circulating levels of pro-inflammatory cytokines such as IL-6, TNF α and the chemokines IL-8, IP-10 (IFN γ -inducible protein 10) and MCP-1 (monocyte chemoattractant protein 1) are also elevated in pre-eclampsia [49].

Inflammation compared with angiogenic imbalance hypothesis

Temporal relationship

According to Hill [50], causation can be determined using the temporal relationship, which implies that the cause of a disorder must precede its clinical signs. Numerous studies have shown that maternal circulatory sFlt-1 is elevated as early as 5–10 weeks before the onset of pre-eclampsia [23,51,52] and sEng is elevated as early as 11–13 weeks of

gestation before the development of pre-eclampsia [53–58]. Furthermore, a decrease in urinary PIGF also precedes the onset of pre-eclampsia [12]. Collectively, these observations provide evidence of the cause and effect relationship between the angiogenic imbalance theory and pre-eclampsia.

In contrast, neutrophil activation [38,59] and elevation of Th1 pro-inflammatory cytokines [49,60–62] have been shown to occur at the time of diagnosis of pre-eclampsia. In addition, a prospective nested case control study and a longitudinal study revealed that, at 18 weeks of gestation, the levels of inflammatory parameters including TNF α , IL-6 and IFN γ were not elevated in women who later developed pre-eclampsia compared with healthy controls [63,64]. The lack of a temporal relationship between the inflammatory cytokines and the maternal syndrome of pre-eclampsia puts into question the long-held concept that pre-eclampsia is caused by ‘excessive inflammation’.

Interestingly, the study by Lynch et al. [65] suggested that early activation of the complement system in pregnancy elevates the concentrations of circulating complement-activated fragment Bb that could be associated with the subsequent development of pre-eclampsia, independent of the release of anti-angiogenic factors [43]. However, given the heterogeneous nature of pre-eclampsia and the small number of pre-eclamptic patients in this study (32 patients who developed pre-eclampsia), additional studies are needed to evaluate the relative implications of complement activation in the pathogenesis of pre-eclampsia.

Strength of association and consistency

Compelling and consistent data from clinical studies conducted in various centres have confirmed that circulating levels of sFlt-1, sEng and PIGF gave the highest strength of association with the clinical manifestation of early-onset pre-eclampsia, satisfying the two criteria of causation: strength of association between the cause and the disease and the consistency of association in different settings [23,51,66,67].

In contrast, clinical data vary greatly with respect to differences in specific level of inflammatory cytokines between normal pregnant women and pre-eclamptic women. Jonsson et al. [68] examined serum levels of 20 different cytokines and found significant differences in only two, IL-6 and IL-8, between pre-eclamptic and normal pregnant women. Sharma et al. [69] observed similar increase in serum IL-6 and IL-8 levels, but also found a significant increase in serum TNF α and a decrease in IL-10 in pre-eclamptic pregnant women compared with normal pregnant women. Additional studies have found increases in placental and/or peripheral blood levels of the inflammatory cytokines IL-2, IL-12, IL-15, IL-18 and IFN γ in pre-eclamptic pregnant women [69–71]. In contrast, Freeman et al. [72] observed that the serum levels of IL-10 and TNF α are increased from the first to the third trimester in healthy control pregnancies to nearly the same degree as in women with pre-eclampsia [72]. The inconclusive and contradictory findings about the inflammatory cytokines challenge the role of inflammation as the main cause of pre-eclampsia. Furthermore, with the

exception of a few studies [73,74], many studies have failed to find a strong and consistent strength of association between the increase in inflammatory status and the clinical signs of pre-eclampsia, weakening the role of inflammation as the cause of pre-eclampsia.

Dose-response relationship

To date, the only cure for pre-eclampsia is the delivery of the baby and the removal of the placenta. Pre-eclampsia also occurs in molar pregnancies (absence of fetus), indicating that it is the placenta and not the fetus that causes the condition. Increase in placental mass, as in singletons to twins, triplets and quadruplets, also increases the incidence of pre-eclampsia. In twin pregnancies, a 2-fold increase in the level of sFlt-1 and an increase in the sFlt-1/PIGF ratio were observed [75]. In a secondary analysis of the NICHD (National Institute of Child Health and Human Development) MFMU (Maternal–Fetal Medicine Unit) trial of aspirin to prevent pre-eclampsia in high-risk pregnancies, the odds of developing pre-eclampsia were significantly increased among women with multiple placenta for each 2-fold elevation in sFlt-1, sEng and the ratio of angiogenic factors, and significantly decreased for each 2-fold elevation in circulating PIGF between 7 and 26 weeks of gestation [76]. Importantly, the fractional excretion of angiogenic factors was significantly higher in women with severe pre-eclampsia compared with those with mild pre-eclampsia or pregnant controls [77], demonstrating that the increase in sFlt-1 is directly proportional to the severity of the disorder. It is also worth noting that sFlt-1 can only induce hypertension and proteinuria in mice when its levels reach above a certain critical threshold [24], supporting the theory that women with fetal growth restriction, despite having elevated levels of sFlt-1 compared with controls, do not exhibit signs of hypertension or proteinuria [78]. These studies clearly demonstrate the dose–response relationship between the level of anti-angiogenic factors and the incidence and severity of pre-eclampsia. To our knowledge, such a relationship has not been established between pro-inflammatory cytokines and pre-eclampsia.

Specificity and experiment

Animal studies provide strong evidence linking sFlt-1 and sEng to the pathogenesis of pre-eclampsia [10,11,25,79–86]. In pregnant rats, sEng acts synergistically with sFlt-1 to induce endothelial dysfunction, hypertension, severe proteinuria and HELLP syndrome [11]. Furthermore, neutralization of sFlt-1 below a critical threshold using VEGF eliminates the signs of pre-eclampsia in mice [24,84]. In addition, Ahmad and Ahmed [26] showed previously that immunoprecipitation of sFlt-1 from placental conditioned medium of pre-eclamptic women restored angiogenesis as demonstrated by *in vitro* tube formation. Following on from this concept, recently, an elegant study by Thadhani et al. [87] showed that the extractions of sFlt-1 from the plasma of pre-eclamptic patients by apheresis (using a negatively charged dextran sulfate cellulose column to which positively

charged sFlt-1 is adsorbed), resulted in an approximate 35% reduction in circulating sFlt-1. As a consequence, this was accompanied by a reduction and stabilization of maternal blood pressure leading to an increase in gestational age in two patients by 16 days (for singleton pregnancy) and 19 days (twin pregnancy) respectively. Collectively, these separate studies fulfil the criteria of specificity and strengthen evidence for the role of anti-angiogenic factors as the likely candidates for the pre-eclampsia.

Several studies have attempted to show that inflammation itself causes the clinical signs of pre-eclampsia. For example, Faas et al. [88] showed that infusion of a single dose of endotoxin to pregnant rats caused glomerular damage in the kidney, as well as hypertension and proteinuria. In addition, Hayakawa et al. [62], who developed an animal model of immune-mediated abortion and pre-eclampsia due to excessive activation of Th1/Th2 lymphocyte, showed that *in vitro*-activated splenocytes with IL-12 and IL-4 injected into pregnant mice induced hypertension, proteinuria and histological renal changes, but not in controls. In a more recent study, Zenclussen et al. [89] showed that activated Th1-like cells, but not Th2-like cells, injected into mice led to elevated blood pressure, proteinuria and kidney damage exclusively in pregnant mice [89]. In support of these studies, other groups have reported that immunosuppression or inhibition of the inflammatory network improves the aforementioned pathological conditions.

Indeed, in a rat model of pregnancy-induced hypertension, as described previously [90], pharmacological immunosuppression of lymphocytes improved blood pressure and endothelial function [91,92], indicating that prevention of T-cell proliferation may decrease hypertension. In a more recent study, Zhou et al. [93] showed that injection of angiotensin-II type I receptor-agonistic autoantibodies induced pre-eclampsia-like features in mice and the inhibition of IL-6 in these mice inhibited these signs. Despite these studies, the proposal that pre-eclampsia is primarily an inflammatory disorder is somewhat at odds with clinical experience of the use of corticosteroids used to accelerate fetal lung maturation. A prospective double-blind randomized clinical trial of 24 mg of betamethasone given as two 12 mg doses 24 h apart and repeated weekly confirmed the known beneficial effects of this treatment on neonatal outcome, but failed to show any beneficial effect to the mother [94]. This dose of betamethasone is equivalent to 160 mg of prednisolone, which would be regarded as a huge dose of corticosteroid and which would be expected to have major effects in other inflammatory conditions. Moreover, in a similar clinical trial, dexamethasone failed to improve the outcome of women with HELLP syndrome [95].

Coherence and plausibility criteria

According to the Hill's criteria of causation, the association needs to be compatible with existing knowledge. The biology of VEGF helps to validate the role of the anti-angiogenic factor sFlt-1 as a cause of pre-eclampsia. VEGF is critical for endothelial cells homeostasis [96] and plays a crucial

role in the health of fenestrated and sinusoidal endothelium found in the renal glomerulus, brain and liver [97], organs that are severely compromised in pre-eclampsia. VEGF activates both VEGFR (VEGF receptor)-1 and VEGFR-2 to stimulate NO required for angiogenesis [30,31,98–102], which suggests its beneficial role in the regulation of vascular tone and blood pressure [103]. Indeed, exogenously administered VEGF was shown to ameliorate post-cyclosporine-mediated hypertension, endothelial dysfunction and nephropathy [104,105]. Beneficial effects of VEGF have also been observed in animal models of kidney disease [106–108]. Furthermore, cancer patients receiving anti-VEGF therapy [22] or rats treated with VEGF-neutralizing antibody or sFlt-1 [109] exhibit pre-eclampsia-like symptoms, indicating that reduction in VEGF bioavailability is the likely initiator of vascular damage responsible for the main clinical signs of pre-eclampsia.

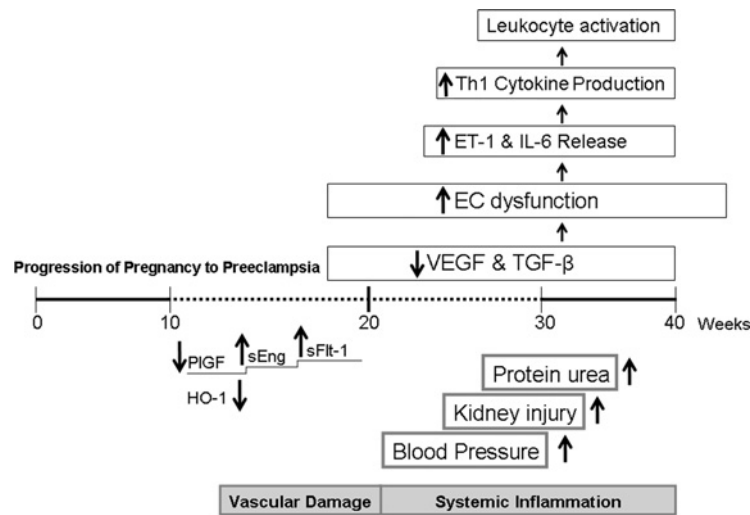
Increases in inflammatory cytokine production have been associated with endothelial dysfunction, increased placenta apoptosis, decreased angiogenesis and kidney abnormalities that are relevant to the pathophysiology of pre-eclampsia [110]. Endothelial cell damage induces the production of ET-1 (endothelin-1) [111,112]. In pregnant mice, ET-1 was shown to cause hypertension, proteinuria and renal damage. Furthermore, IL-6 acts downstream of TNF α to induce the release of ET-1 in mice. In addition, in the presence of autoantibodies from pre-eclamptic women, IL-6 mediates the release of ET-1 from human placental villous explants [93]. These studies validate the original *in vitro* observations of Collino et al. [113], which showed that pre-eclamptic sera induces nephrin shedding from podocytes through ET-1 release by endothelial glomerular cells. These separate studies show an association between inflammation and ET-1 in rodents, and ET-1 may play an important role in pre-eclampsia, but pregnant women with excessive inflammation and high levels of inflammatory cytokines, such as those with infections, do not always develop pre-eclampsia.

Conclusion

Collectively, the studies are important in elucidating the sequence of events involved in the progression of pre-eclampsia and its clinical signs (Figure 1). There is no doubt that both angiogenic imbalance and systemic inflammation are elevated in pre-eclampsia. However, on the basis of the evidence and arguments made above, we advocate that inflammation is a consequence rather than a cause of pre-eclampsia. The most essential criterion of Bradford-Hill is the principle of temporality, which states that when a factor is believed to cause a disease, the factor must necessarily always precede the occurrence of the disease. The absence of temporality between the elevation in the circulating inflammatory markers and the development of the maternal syndrome of pre-eclampsia challenges the concept that excessive inflammation is the cause of pre-eclampsia. In contrast, the increase in circulating anti-angiogenic factors, which precedes the clinical signs of pre-eclampsia, fulfils

Figure 1 | Progression of pregnancy to pre-eclampsia

A schematic diagram of the sequence of events along gestational age leading to the clinical signs of pre-eclampsia. EC, endothelial cell; HO-1, haem oxygenase 1; TGF- β , transforming growth factor β .



the condition of temporality. Another crucial criterion is the dose–response relationship, where increasing the level of exposure to a factor increases the incidence and severity of the disease. Although such a relationship has not been established between pro-inflammatory cytokines and pre-eclampsia, high levels of anti-angiogenic factors have been shown to correlate with increased incidence of the disorder and disease severity, hence satisfying this condition. Furthermore, Hill's experiment criterion states that removal of the cause by an appropriate experimental regimen should ameliorate the condition. Indeed, compelling evidence supporting a role for anti-angiogenic factors as the leading cause of pre-eclampsia has recently been shown in a clinical study, where removal of circulating sFlt-1 from pre-eclamptic patients significantly improved the clinical outcome [87]. In contrast, treatment with high doses of corticosteroids failed to ameliorate the maternal outcome in pre-eclampsia despite suppressing inflammation.

Although inflammation may not be the cause of pre-eclampsia, it may enhance the pathology of the disorder in the presence of the anti-angiogenic factors. A recent study demonstrated that sFlt-1 act synergistically with pro-inflammatory mediators to activate endothelial cells compared with endothelial cells treated with TNF α alone [114]. Treatment with sFlt-1 or VEGF-neutralizing antibodies or blockade of VEGFR-1 and VEGFR-2 reduced the phosphorylation of Akt and eNOS leading to reduced NO bioavailability and increased endothelial dysfunction [114]. Furthermore, a recent study demonstrated that adenoviral overexpression of sFlt-1 in endothelial cells resulted in substantial inhibition of eNOS phosphorylation and NO production as well as a decrease in VEGFR-

2 phosphorylation in pre-eclamptic placenta [27]. These separate results provide additional support for the original hypothesis put forward by Ahmed in 1997 [9] that lack of VEGF and NO bioavailability is responsible for the endothelial dysfunction in pre-eclampsia.

Whereas inflammation itself may not be the leading cause of pre-eclampsia, it should also be recognized that pregnant women with risk factors associated with chronic inflammation (obesity, pre-pregnancy hypertension, diabetes mellitus and dyslipidaemia) are more likely to develop pre-eclampsia [115–118]. Furthermore, patients with autoimmune diseases, particularly systemic lupus erythematosus and antiphospholipid syndrome, have increased risks of developing pre-eclampsia [119,120]. The possibility that inflammatory mediators may have local autocrine or paracrine effects, which can lead to amplification of the anti-angiogenic factors' effects, cannot be ruled out at this stage.

In conclusion, we hypothesize that inflammation is not a major contributor in the development of pre-eclampsia. In the interest of finding an effective treatment, our focus should be to target development of therapies based on the angiogenic [28,121] and cytoprotective [3] theories, which seem more promising.

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