

REVIEW ARTICLE

Pre-Eclampsia: Is It All In The Placenta?

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Submitted: 6 March 2007

Accepted: 1st December 2007

Abstract

Hypertensive disorders of pregnancy complicate almost 7 – 10 % of all pregnancies. The dyad of hypertension and proteinuria after 20 weeks of gestation is referred to as pre-eclampsia. It is a major cause of maternal morbidity and mortality and is also associated with increased perinatal problems. Despite intensive research over the years the exact cause of pre-eclampsia remains unknown. Nevertheless, information gleaned from published studies point to the placenta as the probable pathogenetic focus of pre-eclampsia, as the disease usually resolves within 24 – 48 hours after delivery of the placenta. Although the precise involvement of the placenta in pre-eclampsia remains unclear there are indications that the trophoblastic invasion of the uterine spiral arteries is abnormal in women who develop pre-eclampsia. This impaired invasion leads to decreased placental perfusion and ultimately to placental hypoxia. The distressed or ischaemic placenta then secretes a factor(s) into the maternal circulation, which cause/s widespread endothelial cell dysfunction characterized by vasospasm, activation of coagulation system and organ ischaemia. The cause of the defective cytotrophoblastic invasion of the spiral arteries and the link between placental ischaemia and generalized maternal endothelial dysfunction remain unknown. Although the placenta appears to have a major role in the pathogenesis of pre-eclampsia, evidence also suggests that factors like maternal genetic predisposition, dietary, environmental and behaviour, which surface during the stress of pregnancy might also be involved in the development of pre-eclampsia. It is known that not all women with poor cytotrophoblast invasion develop pre-eclampsia and not all women with preeclampsia show poor cytotrophoblast invasion. Over the years, a number of potential risk factors associated with the development of pre-eclampsia are being recognized and it might be appropriate now to develop some preventative strategies based upon the available information.

Keywords: *pre-eclampsia, placenta, medical sciences*

Introduction

Hypertensive disorders of pregnancy complicate almost 7 – 10 % of all pregnancies. The dyad of hypertension and proteinuria after 20 weeks of gestation is referred to as pre-eclampsia. It is a major cause of maternal morbidity and mortality and is also associated with increased perinatal problems. In spite of the intensive research over the years, the exact cause of pre-eclampsia remains unknown. Numerous causes have been proposed leading some to aptly refer to it as a “disease of theories”. Nevertheless, considerable progress has been made towards the elucidation of a number

of placental and maternal abnormalities that are associated with this disorder.

Historically, the first report of this disorder dates back to nearly 2000 years when Celsus reported an account of seizures in pregnant women that abated after delivery (1). This abnormality was given the name “eclampsia”, which in Greek means “lighting”, to describe its rapid and unexpected appearance. Sometime during the middle of the 1800s, examination of urine for protein in women with eclampsia revealed severe proteinuria that antedated the seizures. In the latter part of the 1800s, when it became possible to measure blood pressure with a sphygmomanometer, it further

became apparent that eclamptic women also had high blood pressure, and like proteinuria this also antedated the seizures. As proteinuria and hypertension antedated eclampsia, the term "pre-eclampsia" was applied to the development of hypertension and proteinuria during gestation. Today the term pre-eclampsia is used when there is raised blood pressure and proteinuria after 20 weeks of gestation. The mechanisms responsible for these are still unclear.

Placenta and pre-eclampsia

Pre-eclampsia-eclampsia is not a consequence of raised blood pressure or proteinuria *per se*, rather these are markers of multi-organ dysfunction in the mother. Women with pre-eclampsia seem to show disturbances in vasomotor activity, plasma volume and the coagulation system, which have been attributed to endothelial cell dysfunction or activation. The role of endothelial cells in the pathogenesis of pre-eclampsia is indicated by (i) the presence of high circulating levels of von Willebrand factor (2,3), (ii) morphologic evidence of endothelial cell injury e.g. glomerular endotheliosis (4), which is often seen in eclampsia but found in no other forms of hypertension, (iii) high circulating levels of cellular fibronectin (5, 6), (iv) high circulating levels of endothelins (7,8), (v) disturbances in the total plasminogen activator (tPA) and plasminogen activator/inhibitor-1 balance (9), (vi) altered prostacyclin (PGI₂)/thromboxane (TXA₂) balance (10) These morphologic and functional changes of the endothelial cells can be held directly responsible for triggering arterial vasospasm, increased thrombocyte aggregation, and increased capillary permeability that lead to hypertension, proteinuria, oedema and sometimes thrombocytopenia and hypoperfusion of organs (HELLP syndrome).

What causes the endothelial dysfunction remains a speculation. It has been proposed that some factor/s originating from the distressed placenta might be responsible for this disturbance or enhanced endothelial function. That the abnormality may indeed be in the placenta, to begin with, is supported by numerous clinical, biochemical and morphological observations, and possibly from some animal studies too. It is well known that the pathophysiological and pathological changes abate after delivery of the fetoplacental unit. It is also known that the frequency of pre-eclampsia is increased in hydatidiform mole indicating that the presence of the fetus is not necessary (11,12). There has also been a report of pre-eclampsia in a woman with abdominal pregnancy, implying the irrelevance of the decidua

or a distended uterus (13). The precise nature of the abnormality in the placenta or what is responsible for it however remains unclear. Nevertheless, there is evidence to suggest placental hypoperfusion and consequent ischaemia (14) probably secondary to poor cytotrophoblast invasion of the uterine wall. It is known that cytotrophoblastic invasion, though generally continuous, occurs in two stages i.e. during the first two weeks of gestation and then between 12 and 20 weeks of gestation in normal pregnancy. During this time there is invasion of the maternal spiral uterine arteries by the extravillous cytotrophoblasts. The invasion extends from the intervillous space up to the inner third of the myometrium (15,16). There is evidence that in women with pre-eclampsia the invasion by the trophoblast is defective where it remains limited to the decidual portion of the spiral arteries, with the myometrial segments maintaining their smooth muscle layer (17,18,19). These vessels have diameters that are only 40 percent of those vessels of normal pregnancies (20). Some spiral arteries are also atherosclerotic (21). What causes the defective placentation is not apparent but there, reportedly, is a failure of the invading cytotrophoblasts to express the necessary invasive and adhesive properties or characteristics and signal molecules that are required for proper placentation, cytotrophoblast differentiation, invasion, angiogenesis and vasculogenesis. In normal pregnancy, placental cytotrophoblasts that invade the uterus downregulate the expression of adhesion molecules like Ecadherin and integrin $\alpha 6\beta 4$ and $\alpha V\beta 6$ that inhibit invasion and up regulate receptors like $\alpha 1\beta 1$, $\alpha V\beta 3$ and VE cadherin that promote invasion (22,23). In pre-eclampsia however, cytotrophoblasts fail to differentiate completely and continue to express Ecadherin, integrin $\alpha 6\beta 4$ and $\alpha V\beta 6$. They also fail to up-regulate the expression of $\alpha 1\beta 1$, $\alpha V\beta 3$ and VE cadherin, vascular adhesion molecule (VCAM-1) and platelet endothelial adhesion molecule-1 (PECAM-1) (24,25). The reason for the failure of the cytotrophoblast to differentiate and for placentation to occur satisfactorily is unknown.

In addition to these molecules that aid invasion, there are probably also a number of other molecules that are required for vasculogenesis and angiogenesis. One potential group consists of the *Eph* receptors and their ligands known as *Ephrins*. Their potential involvement in vascular patterning was first suspected when deletion of either EphB4 receptor or its primary ligand, ephrin B2, resulted in a general failure in angiogenic remodeling of the primary vascular plexus and subsequent embryonic lethality at mid-gestation in mice (26). Our

preliminary investigations into the expression of these molecules in normal and pre-eclamptic placentae revealed no expression of the ligand Ephrin B2 in pre-eclamptic placentae ranging in gestation from 26 to 40 weeks (27). It is believed that the poor angiogenesis and vasculogenesis that ensue are responsible for the distress in the placenta. In fact administration of sFlt-1 (an inhibitor of angiogenesis) to pregnant rats has been found to produce hypertension, proteinuria and glomerular endotheliosis (28). The reason for the altered or disturbed expression of these signaling molecules that are necessary for proper implantation of the placenta remains unclear. Recent report suggests of a lack of downregulation of transforming growth factor beta 3 (TGF-beta3) in pre-eclamptic placentae (29). TGF-beta3 is produced by the placenta very early on during gestation and it inhibits trophoblast differentiation. Its level begins to fall after about ten weeks of gestation. Why there is a failure to down regulate TGF-beta3 and what its role is in the impaired placentation in pre-eclampsia is unclear.

In addition to the failure to down-regulate TGFbeta3, placental hypoxia might also contribute to this defect. Evidence from experiments in vitro suggests that cytotrophoblast differentiation is significantly influenced by hypoxia. When cytotrophoblasts were cultured in hypoxic conditions (2% oxygen) they continued to proliferate without proper differentiation. However, when these cytotrophoblasts were cultured in 20% oxygen they stopped proliferating and differentiated normally (29,30). It appears that ischaemia or hypoxia during the second wave of invasion might restrict the development invasive properties by the cytotrophoblasts, consequently affecting the invasion of the myometrium by some segments of the placenta. In addition to poor angiogenesis and vasculogenesis, there is also a possibility that the hypothesized poor placental perfusion might be due to an imbalance of vaso-active factors in the placenta. The placenta lacks neural innervation and blood flow is principally regulated by humoral factors. An imbalance of these in favour of vasoconstrictors might compromise blood flow to and also in the placenta. Numerous vaso-active factors have been identified, some of which include the products of the renin-angiotensin system, kallikrikinin-kininogen system, endothelins, nitric oxide, catecholamines, and vasodilatory and vasoconstrictive eicosanoids. Our observations of these have highlighted the existence of numerous abnormalities in some of these factors in the preeclamptic placentae. For example, placentae from women with pre-

eclampsia were found to have significantly higher levels of prorenin and renin (31), leptin (32) and endothelin-1 (33), and significantly lower levels of kininogen (34), indicating a probable imbalance between vasodilator and vasoconstrictor factors.

Apart from an imbalance between vaso-active factors, there also exists a possibility of the role of immune maladaptation in cytotrophoblast invasion. This possibility is supported by the presence of immunopathology in women with pre-eclampsia where immune complex deposition in the spiral arteries, placenta, kidney and liver have been observed. There is also evidence of increased circulating immune complexes and the presence of acute atherosclerosis in women with pre-eclampsia (35). Whether the immune maladaptation is due to poor maternal desensitization or due to some other factor is unclear. It has however been observed that in normal pregnancy the cytotrophoblasts, which invade the decidua suppress the expression of MHC Class II antigen HLA-A and instead express a nonclassical class I antigen HLA-G. Some of the cytotrophoblasts from women with pre-eclampsia have been reported to be devoid of HLA-G (36,37).

In addition to this, there is also circumstantial evidence that supports the role of immune maladaptation in pre-eclampsia. It is known that a previous pregnancy or abortion by the same father is associated with a lowered incidence of preeclampsia (38). Furthermore, the protective effect of multiparity is lost with a change of male partner (39,40,41) although an earlier study had failed to show this (42). This also suggests that pre-eclampsia may be a problem of primipaternity rather than primigravidity. Moreover, the length of exposure to sperm and cohabitation before pregnancy correlate negatively with pre-eclampsia (43,44). Interestingly, in a couple of isolated studies the incidence of intraoral ejaculation before pregnancy has been observed to be somewhat higher in normal pregnant women when compared to women with pre-eclampsia (45,46). Women using barrier contraceptives have twice the likelihood of developing pre-eclampsia (47,48). Artificial donor 10 insemination has been shown to be associated with a higher risk of pre-eclampsia (49). These observations collectively suggest that a longer exposure to the partner's sperm before pregnancy decreases the maternal immune sensitivity to the fetal allograft, and that in preeclampsia this normal tolerance process of the fetal allograft has not developed completely. Evidently fewer than 4 months of cohabitation among users of barrier methods for contraception is associated with increased risk for pre-eclampsia (50).

Information to date suggests that there exists some abnormality in the placenta, which might be due either to an imbalance in the levels of vaso-active factors or to immune maladaptation, and this abnormality is responsible for the maternal syndrome. To explain this disorder, a two compartment model has been proposed. It hypothesises that to begin with there exists an abnormality in the placenta possibly due to an imbalance in vasoactive factors or immune maladaptation that results in poor cytotrophoblast invasion and consequently placental insufficiency. The distressed placenta then releases some factor/s, which crosses the maternal placental barrier and causes endothelial dysfunction in the mother. The exact nature of this factor/s has not been identified but a number of them have been implicated. The proposed agents include syncytiotrophoblast microvillus membranes shed into the maternal circulation (51), interleukin-1 and 6 (52), tissue necrosis factor- α (53), and VCAM-1 (54), elastase produced by activated neutrophils in the deciduas and released into the maternal circulation (55), neurokinin B (56), AT1 autoantibodies (57) and placental renin (58). In addition to these, there is also a possibility of the involvement of free radicals and lipid peroxides released from the distressed placenta in pre-eclampsia. Owing to hypoxia, ischaemia and infarction of the placenta, there is a possibility that uric acid production may be higher in placenta from pre-eclamptic women. Xanthine oxidase activity generates reactive oxygen species like super oxide and hydrogen peroxide (59,60). Circulating levels of lipid peroxides have been observed to be higher in women with pre-eclampsia (61). Mitochondria from pre-eclamptic placenta are larger and evidently generate more lipid peroxides than those from normal placenta (62). In addition to the production of more oxidants in the form of superoxides there is also evidence for decreased antioxidant activity in the sera of pre-eclamptic women (63, 64). It is unclear if the decreased antioxidant activity is due to decreased production of antioxidants or an increased production of oxidants. Tissue vitamin E levels, activities of Cu-Zn Superoxide dismutase and glutathione peroxidase are lower in pre-eclamptic placenta (65). It is possible the excess free radicals and lipid peroxides might be responsible for the maternal endothelial activation or dysfunction.

Although existing evidence consistently points to the placenta having a major role in the pathogenesis of the maternal syndrome, there nevertheless are indications that seem to suggest a role of some yet to be identified genetic, maternal, and even environmental factors, that might surface

during the stress of pregnancy in some instances of pre-eclampsia. Maternal predisposition, for example, might also account for the maternal symptoms, and consequently affect the placenta and the developing fetus. In this regard it is known that not all cases of pre-eclampsia reveal an abnormal placentation or cytotrophoblast invasion or for that matter placental hypoperfusion. Moreover, not all women with poor cytotrophoblast invasion go on to develop preeclampsia e.g. in some cases of IUGR. In addition, the biochemical abnormalities reported by us, although were significantly different when examined by groups; they were however not present in every pre-eclamptic placenta, although a clear diagnosis of pre-eclampsia was evident in all the cases studied. There was a tendency for slight overlap between values. Furthermore, a higher incidence of preeclampsia in women born of eclamptic pregnancy, and in the first pregnancy in sisters, indicate the presence of some maternal predisposition (66,67, 68). The knowledge that the incidence of preeclampsia is higher in the first pregnancy than subsequent pregnancies and the evident discordance between identical twins (69), no doubt, weakens the role of genetic or familial disposition somewhat. Interestingly, women with blood group AB are somewhat more susceptible to pre-eclampsia (70). Pre-existing hypertension, diabetes mellitus, increased insulin resistance, and increased blood homocysteine increases the risk of pre-eclampsia. Once again interestingly, these are also risk factors for other endothelial diseases like atherosclerosis and the late complications of diabetes mellitus. Even a strong family history of aggregate cardiovascular risk has also been found to increase the likelihood for developing pre-eclampsia and transient hypertension during pregnancy (71).

That there may be other factors outside of the placenta, such as diet and environment that might Harbindar Jeet Singh also be involved is supported by the finding that the prevalence of pregnancy-induced hypertension is higher in populations in areas where calcium consumption is lower, and lower in populations given calcium supplementation during pregnancy (72,73,74). The role of calcium in the pathogenesis of PIH and pre-eclampsia is unclear but numerous small scale studies have indicated a reduced incidence of PIH and preeclampsia in populations given calcium supplementation during pregnancy (75, 76). One large-scale study however found no significant effects of calcium supplementation on preeclampsia, pregnancy-induced hypertension, or any adverse outcomes for that matter, when given to healthy nulliparous women (77). The authors

however do concede that in populations with a low dietary calcium intake, calcium supplements might have a role in preventing pre-eclampsia. A recent report in the Cochrane database systemic review concludes from studies to date that calcium supplementation during pregnancy appears to almost halve the risk of pre-eclampsia (78). Significant disturbances in calcium homeostasis and possibly also magnesium in women with pre-eclampsia and pregnancy-induced hypertension have been reported (79, 80, 81, 82). Although calcium status is rarely assessed during pregnancy, it is known that serum total calcium falls during normal pregnancy, accompanied by a fall in urinary calcium excretion (83). This usually occurs during the latter part of the second trimester and during the third trimester of pregnancy when there is increased fetal accretion of calcium. The fall in serum calcium is however somewhat greater in women with PIH and preeclampsia. A recent study also found significantly lower levels of 25(OH) D in women with preeclampsia (84). They report of a monotonic dose-response relation between serum 25(OH) D concentrations at <22 weeks and risk of preeclampsia. A 50-nmol/l decline in 25(OH) D concentration evidently doubled the risk of pre-eclampsia.

In addition to genetic and dietary factors, there may also be environmental and behavioural factors that might affect the risk of pre-eclampsia. For example, a recent report from 12 hospitals in America found that the incidence of pre-eclampsia decreases during the summer months in white women but not in black women (85). In addition, IgG seropositivity for *Chlamydia pneumoniae* is more common among women with pre-eclampsia (86,87). The incidence of pre-eclampsia is reportedly lower in women who smoke (88).

Clearly, the aetiology and pathogenesis of preeclampsia still remains an enigma and there is a continued need for further study and the realisation that pre-eclampsia might not have a single aetiology, and that it might be a heterogeneous entity.

While all the research endeavours continue to unravel the precise aetiology of this disorder, it might be appropriate now to formulate and implement some actions, based on what we know so far, that could help reduce or prevent the incidence of pre-eclampsia. Until such time that we fully understand the causes of pre-eclampsia, we should continue to explore ways to also help prevent or minimize the influence of some of the suspected risk factors in this disorder. One approach that might be useful is the 'risk factor approach' that has been successfully implemented in the reduction of cardiovascular

disease. Of consideration here would be the use of calcium supplements, possible also antioxidants, planning of pregnancy and even physical activity. Of these, calcium supplementation appears by far the most promising and with less adverse effects. The use of vitamin E supplementation in some instances although has been found to reduce the risk of pre-eclampsia and small-for-gestational age babies, there however seems to be an increased risk of pre-term birth (89). Vitamin C supplementation has produced no significant effect on the incidence of pre-eclampsia (90).

Planning of pregnancy with sufficient prior exposure to the partner's semen might also be worth the consideration. Although the precise duration of exposure before conception has not been determined, but from the little available evidence there is a period of at least 4-6 months might be the minimum required (50).

Although the role of exercise in the reduction in the risk of hypertension and other cardiovascular complications is well documented for the normal population, its role in the prevention of preeclampsia has not been examined. It is known that women who are most physically active have the lowest prevalence of gestational diabetes. Given that preeclampsia, atherosclerosis, and diabetes share a common dyslipidaemia i.e. increased triglycerides, decreased HDL, and increased LDL concentrations, it is proposed that the incidence of pre-eclampsia might also be similarly lower in women who are regularly physically active. It is therefore not unreasonable to propose that all women, particularly those with a family history of cardiovascular diseases, diabetes mellitus and pre-eclampsia should exercise regularly to ensure an adequate level of prenatal fitness, which might help reduce the risk of them developing pre-eclampsia. exercise regularly to ensure an adequate level of prenatal fitness, which might help reduce the risk of them developing pre-eclampsia.

Conclusions

In conclusion, although the pathogenesis of pre-eclampsia still remains an enigma, and evidence points to the major role for the placenta in this disorder, there nevertheless is a lot of convincing evidence that seems to suggest a role for factors like maternal genetic predisposition, dietary, environmental and behaviour that might independently contribute to the development of preeclampsia. It appears that pre-eclampsia is a heterogeneous disorder with multi-factorial aetiology, and we have to keep that in focus when studying this disease. There is a continued need

to explore the role of these factors and a lot has still to be done to be able to have some semblance of understanding of this enigmatic disorder. Nevertheless, while this is being pursued, we need to also seriously consider initiating actions, based on the available information, to prevent or minimize the influence of some of the risk factors that have been associated with preeclampsia. It is possible, attention to diet, family history, planning of pregnancy, and perhaps physical activity might help to reduce the incidence of this disease.

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