Review Article

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Biomarkers for the management of pre-eclampsia in pregnant women

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Pre-eclampsia (PE) is a pregnancy related disorder characterized by hypertension and proteinuria noticeable after 20 wk of gestation. It is a leading cause of maternal and foetal mortality and morbidity worldwide. The aetiology of the disease is unknown, but recent studies have revealed that this disorder appears to originate in placenta and is characterized by widespread maternal endothelial dysfunction. Till date, delivery of placenta is the only cure for the disease. So, there is a need for the identification of highly specific and sensitive biochemical markers that would allow early identification of patients at risk and thus help in providing proper prenatal care. Several promising biomarkers have been proposed, alone or in combination, that may help in predicting women who are likely to develop PE. Maternal serum concentrations of these biomarkers either increase or decrease in PE during gestation. This review focuses on the various biomarkers available and their utility in predicting pre-eclampsia.

Key words Angiogenic factors - biomarkers - NGAL - pre-eclampsia - placenta - proteinuria

Introduction

Pregnancy is commonly viewed as a cooperative interaction between mother and foetus. Since, a combination of maternal and paternal genes form the foetal genotype, a genetic conflict induced by paternally derived factors may exist between mother and foetus during placentation and expression of genes in the foetus dictated by the embryo's development needs. Continuous struggle and exchange of signals take place between them to maintain equilibrium. Any aberration in this equilibrium could result in events like pre-eclampsia (PE)[1]. Pre-eclampsia, a condition prior to eclampsia (Greek word "eklampsis" meaning sudden flashing)[2], is a systemic syndrome characterized by symptoms like hypertension, proteinuria and oedema, often complicated by renal failure, pulmonary oedema and coagulopathy. Consequences of these could be retarded growth of the foetus or mortality preceded by seizures and coma. Pre-eclampsia can occur in early pregnancy termed as "early onset pre-eclampsia" at <34 wk gestation and late onset pre-eclampsia which occurs after 34 wk of gestation. However, endothelial dysfunction is common in both early and late onset,

responsible for the symptoms like proteinuria and hypertension. Failure to control these symptoms would result in foetal prematurity and premature delivery[3]. In developing countries with limited access to health care, pre-eclampsia is a cause for about one third of maternal mortality[4]. Pre-eclampsia and eclampsia are seen in around 4.6 per cent of all deliveries[5] and the neonatal mortality rate is around 43 per 1000 live births in India. Therefore, early detection or prediction of PE is imperative[6] and non-invasive diagnostic methods based on biomarkers hold the promise.

Pathogenesis of pre-eclampsia

Placenta plays a major role in pre-eclampsia. The exact cause for the pathogenesis of pre-eclampsia remains unclear. However, several studies have reported that this condition results due to abnormal placenta rather than the foetus[7]. Pre-eclampsia occurs only in the presence of a placenta and almost always remits after its delivery. As in the case of the hydatidiform mole, the presence of a foetus is not necessary for the development of pre-eclampsia[7]. Similarly, in a case of pre-eclampsia with an extrauterine pregnancy, removal of the foetus alone was not sufficient, and symptoms persisted until the placenta was delivered[8].

In normal placental development the cytotrophoblasts invade the maternal spiral arterioles and transform them from small caliber resistance vessels to high caliber conduit vessels. This initial event begins at the end of first trimester (10-12 wk) and ends by 18 to 20 wk of gestation. During this vascular invasion the cytotrophoblasts differentiate from epithelial phenotype to an endothelial phenotype, a process known as pseudovasculogenesis. During this process, these make a direct contact with maternal blood. This process involves a considerable number of transcription factors, growth factors and cytokines like VE-cadherin and alpha v beta-3 integrins[9]. During pre-eclampsia, the invasive cytotrophoblasts fail to transform epithelial phenotype into endothelial phenotype, instead the invasion of the spiral arterioles is shallow and these remain as small caliber resistance vessel which leads to defective uteroplacental circulation and subsequently placental perfusion worsens[7].

The process of pseudovasculogenesis decreases resistance in blood vessels and thereby increases blood flow to placenta so that it can sustain the growing foetus by providing essential nutrients and oxygen. However, in pre-eclampsia the placenta becomes hypoxic within the intervillous space which might trigger tissue oxidative stress and increase placental apoptosis and necrosis finally leading to endothelial dysfunction and an exaggerated inflammatory response[10].

Angiogenic factors are thought to be responsible for the regulation of placental vascular development. Soluble Fms-like tyrosine kinase -1(sFlt-1), vascular endothelial growth factor (VEGF-1), VEGF-2, placental growth factor (PIGF) and sEndoglin are essential for normal vascular development. Therefore, the loss of endothelial control of vascular development leads to hypertension, increased vascular permeability causes proteinuria and disturbed endothelial expression of coagulation factors resulting in coagulopathy[11] (Figure).

Studies have reported that placenta is also the potential source of circulating inflammatory cytokines as the increased serum levels of interferon gamma-inducible protein (IP-10), monocyte chemotactic protein (MCP-1), intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1) and decreased levels of interleukin-10 are reported in pre-elcamptic women when compared with normal pregnant women[12]. More work is needed to uncover the underlying molecular mechanisms leading to the progression of vasculogenesis to angiogenesis in normal placentation and finally to pseudo-vasculogenesis in abnormal placentation as well.

Risk factors for pre-eclampsia

Pre-eclampsia develops only during pregnancy. The risk factors include *(i)* A family history of pre-eclampsia, *(ii)* usually in first pregnancy, *(iii)* higher for pregnant women younger than 20 and older than 40 yr, *(iv)* obesity, *(v)* common in women who are carrying twins, triplets or more, *(vi)* prolonged interval between pregnancies, *(vii)* women who develop gestational diabetes face a greater risk of developing pre-eclampsia as the pregnancy progresses, and *(viii)* history of certain medical conditions such as chronic high blood pressure, migraine headaches, diabetes, kidney disease, rheumatoid arthritis, urinary tract infections, and periodontal disease during pregnancy increases the risk of pre-eclampsia[13].

Current clinical tools for management of pre-eclampsia

Until recently most work on pre-eclampsia has focused on abnormal placentation, genetic and epidemiologic factors, as well as treatments aimed at slowing the progression of the disease. An elevated uric acid level seems to help in diagnosis of PE[6].

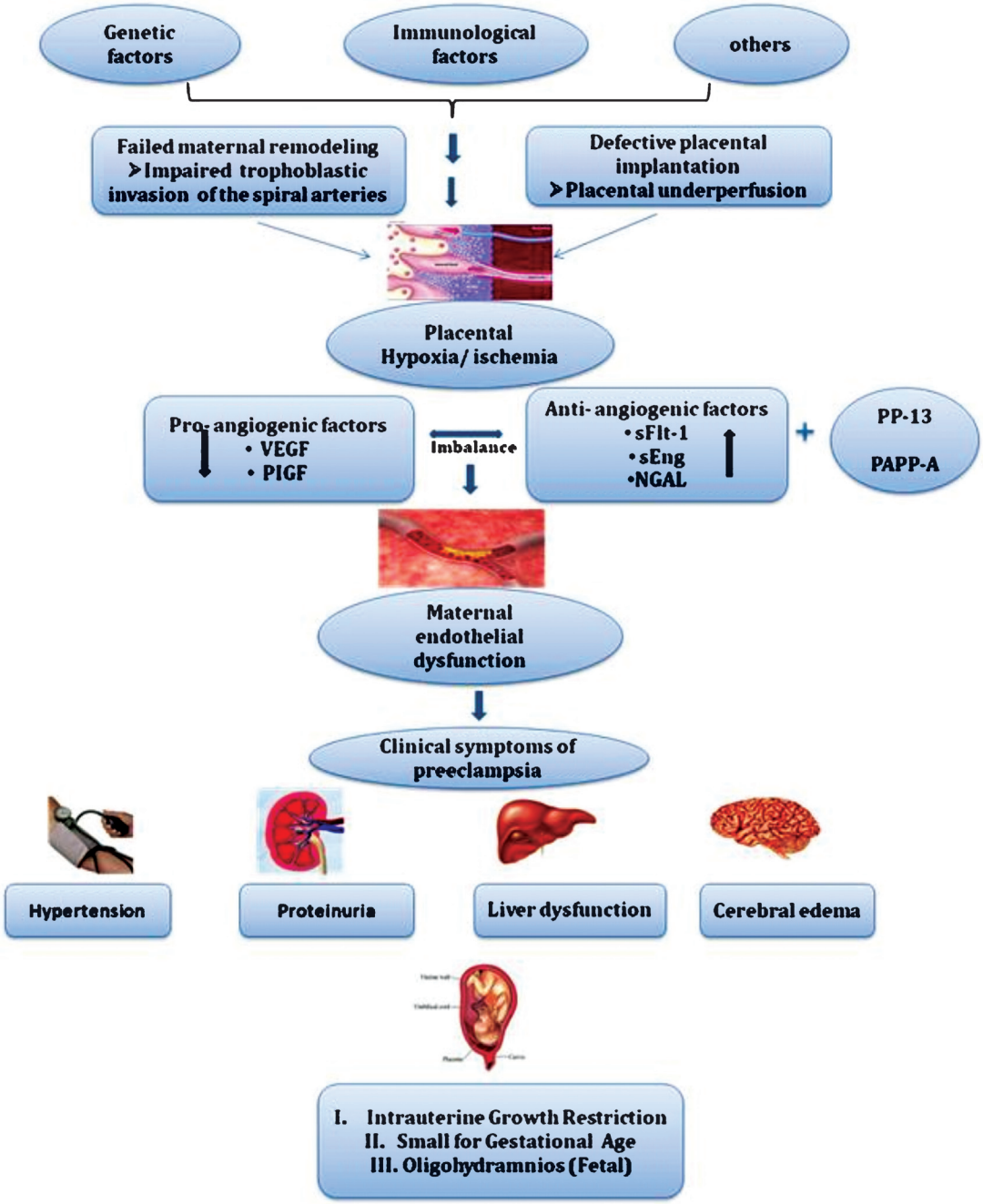

Fig. Flow diagram showing process of pathogenesis of pre-eclampsia.

Doppler studies of brachial artery reactivity in women who have had pre-eclampsia show abnormal endothelial dependent flow-mediated arterial dilation three years after pregnancy[10]. A relation between high resistance uterine artery waveforms in the second half of pregnancy and pre-eclampsia has already been established and persistent notching of the uterine artery Doppler waveform has shown promise as a screening test at 20 and 24 wk[14]. With the introduction of transvaginal ultrasound probes it has been possible to investigate the uterine circulation in early pregnancy with Doppler ultrasound but so far this approach has not been shown to be useful for prediction of utero-placental complications such as pre-eclampsia or the delivery of a small for gestational age baby[14]. However, with the recent advances in 3D Power

Doppler ultrasound qualitative as well as quantitative assessments have become feasible predicting adverse pregnancy outcomes[15].

Proposed laboratory tools for predicting/monitoring pre-eclampsia

Pre-eclampsia affects approximately 2-8 per cent of pregnancies worldwide and is considered as the major cause of maternal and foetal morbidity and mortality[16-18]. In view of the serious consequences of the disorder, efforts are being made to identify effective biomarkers for the risk assessment and disease management.

An ideal biomarker of pre-eclampsia is the one that would allow an accurate prediction during the first trimester as it offers a wide window of opportunity for effective treatment that may help in complete recovery or reduce the severity[19].

Several groups have been investigating biomarkers associated with the pathophysiology of pre-eclampsia, like endothelial dysfunction, general inflammatory response[12,20-22], proteinuria, placental dysfunction as potential diagnostic tools[23]. This review mainly focuses on the various biomarkers available and the recent advances in the utility of these biomarkers in predicting pre-eclampsia.

Vascular endothelial growth factor (VEGF), placental growth factor (PlGF)

Angiogenesis is one of the important processes that contributes to the development of placental vascular system. Among the various angiogenic factors expressed by the placenta, VEGF and PlGF play a very important role[10]. VEGFs are a family of structurally related dimeric proteins whose members include VEGF-A, VEGF-B, VEGF-C, VEGF-D and PlGF. The function of VEGF is to promote sustenance, migration and differentiation of endothelial cells and also to promote vascular permeability. VEGF discharges its function by interacting with VEGFR-2 (also known as Kinase Domain Region), high affinity receptor tyrosine kinases Flt-1 (VEGFR-1) on the placental endothelial cells[24].

Several studies have reported a decrease in VEGF in serum of pre-eclamptic patients. The activity of sFlt-1 (a soluble form of VEGF receptor-1 or sVEGFR-1) which is upregulated in pre-eclamptic conditions can be attributed to this[25]. Free serum sFlt1 is capable of binding with both VEGF and PlGF, thereby neutralizing them, and subsequently decreasing their levels in circulation[25]. A recent study reported decreased production of VEGF by circulating T and natural killer cells in pre-eclampsia, which might contribute to the development of the endothelial dysfunction characteristic of the maternal syndrome of the disease[26]. Although it has been reported that the serum VEGF acts as a promising marker in the prediction of pre-eclampsia[27], most studies could not detect it as the circulating levels of VEGF is as low as <30 pg/ml, which is below the detection limit of many available ELISA kits[18]. This limitation can be overcome if ELISA kits sensitive enough to detect single digit picogram concentration with high reliability are used[18].

Placental growth factors (PlGF) is one of the most important members of VEGF family. Along with VEGF it also plays a key role in angiogenesis and placental vasculature especially during embryogenesis[23]. The major source of PlGF is placental trophoblasts and it is expressed as several different isoforms like PIGF-1, PIGF-2, PIGF-3, and PIGF-4. Unlike VEGF, PIGF binds only to VEGFR-1[28].

The splice variant of VEGFR-1, the sFlt-1, readily neutralizes the PlGF, thereby decreasing its level in serum of women who developed pre-eclampsia[10,23,27]. Studies have shown that in both early and late onset pre-eclampsia, maternal serum levels of PlGF are lower (which could be explained by high sFlt-1 concentration)[23,29].

The PIGF: sFlt-1 ratio has been proposed as one of the best methods for predicting pre-eclampsia before the onset of the disease[23]. The lowering of the PlGF levels during 9-11 wk before the onset of pre-eclampsia, with considerable reduction during 5 wk before the onset of the disease is the critical observation that warrants investigation of the predictive capabilities of PlGF[18,24,27].

Soluble Fms-like tyrosine kinase -1(sFlt-1)

sFlt-1 is an anti-angiogenic soluble form of type -1 VEGF receptor. It results from alternative splicing of Flt-1 receptor mRNA, which is an endothelial receptor for VEGF and PIGF. sFlt-1 consists of an extracellular ligand binding domain of Flt-1, but lacks the transmembrane and intracellular signaling domain. This secretory form circulates freely in the serum where it binds and neutralizes the VEGF and PlGF[11].

A significant rise in serum levels of sFlt-1 has been shown in women who develop pre-eclampsia when compared to control subjects[25]. The serum levels

of sFlt-1 were determined by specific and sensitive ELISA[25]. Importantly, the specific elevation of serum sFlt-1 levels 5 wk before the onset of hypertension and proteinuria only proves the central role of sFlt-1 and impaired VEGF signaling in the development of pre-eclampsia[10]. Baumann *et al*[30] also reported that sFlt1 and sEng levels were increased in the first trimester in women with subsequent late-onset pre-eclampsia and might therefore, prove useful to predict early onset pre-eclampsia.

Levine *et al*[31] reported a mean sFlt-1 value of 4382 pg/ml in women with pre-eclampsia compared to 1643 pg/ml in control group suggesting that higher than normal levels of sFlt-1 are predictive of pre-eclampsia. However, there are reports of poor predictive value and lack of specificity of sFlt-1 in the early stages of pregnancy[23]. Recently, a few variants of sFlt-1, like sFlt1-14 have been discovered[10]. sFlt1-14 level showed an increase in the serum of the patient during pre-eclampsia, thus, questioning the specificity of sFlt-1 in predicting pre-eclampsia[10]. A study conducted in the first trimester Chinese women showed that sFlt-1 levels were normal but PIGF levels increaseed in PE patients[32].

Though several studies have confirmed the role of sFlt-1 in the pathogenesis of pre-eclampsia, keeping in view the above mentioned drawbacks, soluble endoglin and PlGF levels should also be taken into consideration in predicting the pre-eclampsia condition in risk groups[10].

Soluble endoglin (sEng)

Endoglin is a transmembrane glycoprotein with two splice variants, endoglin S and endoglin L[17]. It is highly expressed on the cell membrane of syncitiotrophoblasts and endothelial cells[27]. It acts as a co-receptor for transforming growth factors like, TGFβ1, TGFβ3, and modulates TGF β signaling in angiogenesis and regulates vascular tone[17,27].

Soluble endoglin (sEng) is a truncated form of endoglin and it acts as a potential anti-angiogenic factor by interfering with the binding of TGFβ1 to its receptor, which ultimately affects the production of nitric oxide (NO), vasodilation, and capillary formation by endothelial cells[17]. Recently, the correlation between the increase of sEng serum levels and the severity of pre-eclampsia has been confirmed. The serum levels of sEng are stable throughout in a normal pregnancy, while the concentration of sEng increased (especially during second trimester) in women who develop pre-eclampsia[17].

Studies have shown the development of pre-eclampsia like symptoms in pregnant rats on being transfected with sFlt-1 encoding recombinant adenovirus and sEng encoding recombinant adeno-virus. This has provided strong evidence regarding the potential of sEng as a marker for predicting preeclampsia[17,23].

The serum levels of sEng have been shown to be significantly increased before the onset of disease in women with pre-eclampsia by 9-11 wk and in-term pre-eclampsia (at >37 wk) by 12-14 wk, respectively[31] and effective prediction can be achieved at 11-13 wk gestation[33]. However, the elevated levels of sEng throughout pregnancy was also associated with other forms of pregnancy disorders like SGA (small gestation age), thus limiting the specificity of this marker in predicting PE[27].

Levine *et al*[31] evaluated the potential of sEng in combination with other pro- and anti-angiogenic factors like PlGF, sFlt1 for the prediction of pre-eclampsia. The study implied that the sFlt-1: PlGF ratio and more specifically (sFlt-1+sEng): PlGF was more strongly predictive of pre-eclampsia than were individual markers[34]. However, cohort studies with large study populations are needed to confirm the potential of these biomarkers for diagnosis of PE[27].

Placental protein 13(PP-13)

Placental protein 13 (PP13) is a 32 kDa dimeric protein and it was first isolated from placenta and specially by the syncytiotrophoblast[15] in 1983 by Bohn *et al*[35]. It was subsequently characterized as a member of the galectin super family, whose members are important in placenta implantation and remodel of maternal arteries[19,27]. PP13, which is exclusively produced by placental tissue, possesses a conserved carbohydrate binding domain, to which two proteins Annexin-II and Actin-beta bind. These proteins are considered to play a key role in placentation and maternal artery remodelling respectively[19].

The PP13 levels gradually increase in normal pregnancy. Abnormally low levels of PP13 were found in women who developed pre-eclampsia, when compared to the controls during the first trimester[16,19,27]. The levels of PP13 were found to be high in all risk groups which included pre-eclampsia, IUGR and preterm delivery during 2nd and 3rd trimesters, thereby,

indicating that the measure of serum PP13 during the first trimester can be useful for early prediction of risk of pre-eclampsia[27].

Nicolaides *et al*[19] demonstrated that the combination of serum PP13 levels and uterine artery pulsating index (Doppler ultrasonography) showed a good prediction of patients with the risk of developing pre-eclampsia in the first trimester. Serum PP13 levels were measured using a PP13 ELISA kit and this kit provided straight-forward and reliable results with good sensitivity and specificity[19,27]. Thus, PP13 whether used alone or in combination with Doppler studies, can be considered as a reliable marker in prediction of pre-eclampsia as early as in first trimester, thereby, creating a wide window of opportunity to implement treatment strategies[16,19,27,36].

Pregnancy associated plasma protein-A (PAPP-A)

Pregnancy associated plasma protein-A (PAPP-A), is a 1628 amino acid protease, mainly produced by the placental trophoblasts[27]. It is responsible for the cleavage of insulin-like growth factor binding proteins (IGFBP-4), that play an important role in regulating foetal growth[25].

In a recent study by D'Anna *et al*[37], it was found that the levels of PAPP-A were significantly reduced in the early onset pre-eclampsia while the levels of PAPP-A in the late onset pre-eclampsia did not differ from the control group, thereby concluding that first trimester PAPP-A is not useful in predicting late onset pre-eclampsia. Larger trials are required to confirm these preliminary predictions[25].

Neutrophil gelatinase associated lipocalin (NGAL)

Neutrophil gelatinase associated lipocalin (NGAL), also known as lipocalin-2, siderocalin, uterocalin and 24p3, is a 25 kDa secreted protein that belongs to the family of lipocalins[38,39]. It was first identified as a matrix protein of specific granules of human neutrophils. Its expression is highly upregulated in damaged epithelial cells, during inflammation, neoplastic conditions, cardiovascular diseases, infections and renal disorders[40].

Till date, NGAL is considered as the best and the earliest markers of acute kidney damage, where its presence can be detected in the urine within 2 h following the renal insult[40,41]. In view of both endothelial injury in pre-eclampsia, high blood pressure and kidney impairment characteristics, a recent study demonstrated that the serum levels of NGAL increased at the end of the second trimester in women who subsequently developed pre-eclampsia compared to the control group[37,39,42]. The study was performed using quantitative sandwich enzyme immunoassay technique[39] and the observations for serum NGAL levels at each trimester showed a higher NGAL serum concentration at mid trimester and thus considered useful in predicting pre-eclampsia[43]. The NGAL serum values and their positive correlation with the systolic and diastolic blood pressure and with proteinuria, makes NGAL a reliable biomarker for early prediction of pre-eclampsia[37,39,43].

Most of these studies had limited sample size. There has been no report from India regarding the association of biomarkers to pre-eclampsia. Large cohort studies are required to establish the useful cut-off serum levels, for assessing the specificity and accuracy of these biomarkers for diagnostic purposes and for the management of pre-eclampsia[37].

Conclusion

The past decade has brought exciting advances in our understanding of the pathogenesis of pre-eclampsia. There have been several studies in quest for new biomarkers to diagnose pre-eclampsia. Anti-angiogenic factors like sFlt-1, sEng and pro-angiogenic factors like VEGF, PIGF have shown to be promising biomarkers. Recent reports promise a role of retinol binding protein-4[44] and the cytokine IL-10[45] in the pathogenesis of PE, but their value in the diagnosis and management of PE needs to be established. These biomarkers have certain drawbacks like, lack of high sensitive assay kits, inability to predict onset of the disease during initial stages of gestation, low specificity, lack of prognostic value and other issues.

From a diagnostic standpoint, a recent development in the search for a promising biomarker of pre-eclampsia is the discovery of NGAL. Studies conducted so far showed elevated NGAL levels in the serum samples of pre-eclamptic patients during the first and second trimesters. This correlates well with the endothelial damage that occurs during pre-eclampsia and thus NGAL can be considered as a promising marker in predicting both early and late onset pre-eclampsia. It may be required to combine one or more biomarker with NGAL to increase the precision, and sensitivity for early detection of risk and reliability of using biomarkers for pre-eclampsia. Studies involving larger populations are required to determine the diagnostic

cut-off levels and to assess the specificity and accuracy of NGAL for the management of pre-eclampsia in Indian women.

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