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Review Article

Physiology Section

Role of Biomarkers in Early Detection of Preeclampsia

MANISHA KAR

ABSTRACT

Preeclampsia (PE) is a pregnancy-related, potentially life threatening condition. The incidence of PE has increased in the past decade, which has been attributed to various predisposing factors. Abnormal placentation is central to the evolution of this disease process. However, the triggering factor for this is still unknown. Interestingly, intense research done in this arena has unveiled the names of some important biomolecules which play important role in the vasculogenesis of the early placenta, namely, vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) and their antagonists, namely, soluble fms-like tyrosine kinase 1 (sFlt-1, also known as sVEGFR1), and soluble endoglin (sEng). Besides these, Renin Angiotensin System (RAS) was also implicated in this disease process. The roles of immune factors, genetic factors have been stressed from time to time. More novel approaches made, have shed light on the upcoming biomolecules. All these endeavours are directed to diagnose PE as early as possible, which is a real challenge. Question remains whether a single set parameters could diagnose a disease entity which is as complex as PE. Therefore, it is imperative to design feasible, predictive test-set utilizing multiple biomarkers.

Keywords: Preeclampsia, Potential biomarkers, Early prediction

INTRODUCTION

Preeclampsia (PE), a pregnancy-related potentially life threatening condition, which affects 3–5% of pregnancies, has been recognized clinically since the time of Hippocrates. However, its aetiology and pathophysiology remain enigmatic [1]. Clinically, it is characterized by sustained new-onset hypertension, oedema and proteinuria, which typically develop after 20 weeks of gestation [2]. Overall, 10-15% of all direct maternal deaths are associated with PE in low and middle income countries [3]. Significant morbidity and mortality occur in both mothers and foetuses. The incidence of PE has increased in the past decade. This may be related to increased prevalence of predisposing factors, namely maternal age, chronic hypertension, diabetes, pre-pregnancy obesity, and multiple births [4]. Recently, it has been hypothesized that an altered immune response which leads to abnormal placentation, followed by reduced placental perfusion in early pregnancy, causes syncytiotrophoblast ischaemia and shedding of this layer. Excessive shedding of syncytiotrophoblast layer causes extensive damage of vascular endothelium. This in turn, results in an exponential production of multiple cytokine and growth factors, leading to the clinical manifestations of PE [5]. How the immune response can activate the cascade process is still unknown, but it has been proposed to act in synergy with additional exacerbating factors like predisposing maternal and ambient factors [6].

The diagnosis of PE remains a challenge, because it relies on non-specific signs of the disease. Recent research done, has endeavoured to reveal the names of a number of biomolecules which could be potential candidate to be used in diagnostic procedures. The biomarkers of PE belong to various categories and many novel biomolecules are being added to the list each passing day. In the present review, few selected, promising biomolecules have been discussed, which have the potential to be used in clinical diagnostics of PE [Table/Fig-1].

Category	Name of biomarker
Angiogenic markers	Pro-angiogenic: VEGF, PlGF Anti-angiogenic: sflt-1, sEng
Renin Angiotensin System related	Auto antibodies against angiotensin II type 1 (AT_1) receptor
Immunological markers	PP-13, PAPP-A
Metabolic marker	Visfatin
Endocrine markers	Activin A, Inhibin A

[Table/Fig-1]: Potential biomarkers for early detection of preeclampsia
VEGF: Vascular Endothelial Growth Factor, PlGF: Placental Growth Factor, sflt-1: soluble fms-like tyrosine kinase 1, sEng; soluble Endoglin, PP-13: Placental protein-13, PAPP-A: Pregnancy associated plasma protein-A

Angiogenic markers

Angiogenic factors and their receptors are important regulators of placental vascular development [7]. The most widely studied serum markers for PE are vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) and their antagonists, namely, soluble fms-like tyrosine kinase 1 (sFlt-1, also known as sVEGFR1), and soluble endoglin (sEng) [7].

Anti-angiogenic marker

Soluble FLT-1 (sFLT-1)

sFlt-1 is a truncated splice variant of the membrane-bound Flt-1. It circulates freely in the serum, where it binds and neutralizes VEGF and PlGF. Several studies have demonstrated the association between increased sFlt-1 and PE [8, 9]. sFlt-1 levels begin to rise as early as 5 weeks before the onset of PE and they remain elevated, as compared to those seen in unaffected women [10]. The sFlt-1 levels are correlated directly with severity of disease and they are inversely related with time to onset of proteinuria and hypertension [11].

Soluble Endoglin (sEng)

sEng is a truncated form of receptor for $TGF\beta_1$ and $TGF\beta_2$. sEng is a potential anti-angiogenic factor which interferes with binding of TGFβ1 to its receptor, and which thereby affects production of nitric oxide (NO), vasodilation, and capillary formation by endothelial cells *in vitro* [12]. In normal pregnancy, levels of sEng in serum fall

between first and second trimesters. However, it has been reported that sEng is elevated in second-trimester maternal serum in patients who are destined to develop severe preeclampsia [7].

Pro-angiogenic markers: Placental Growth factor (PlGF), Vascular Endothelial Growth factor (VEGF)

Angiogenesis is the key process for proper development of placental vascular system. Both VEGF and PlGF, one of the variants of VEGF, are key players in this process. The level of free PlGF in blood is found to be decreased in PE, though normally, its concentration increases during first 30 weeks of gestation [7]. PlGF level in urine can be measured easily, as it is readily filtered through the kidney [13]. However, the low concentration of free VEGF in PE is difficult to measure by the commercially available ELISA kits [14]. Therefore, the decreased level of PlGF and sFlt-1/PlGF ratio seen during mid-gestation have been proposed as a predictive model for development of clinical PE [12].

It is evident that the fine balance between angiogenic-antiangiogenic factors is disturbed during PE. However, the exact underlying molecular basis is not yet clear. Extensive research done in this arena has pointed towards the interplay between multiple players, namely, alterations in the renin-angiotensin-aldosterone axis, oxidative stress, immune maladaptation and genetic factors.

Biomolecules related to Renin Angiotensin System: Auto antibodies against angiotensin II Type 1 (AT_1) receptor Renin Angiotensin System (RAS) is one of the most important regulators which controls blood pressure, especially for long term control of blood pressure. In addition, RAS has been implicated in vascular remodelling, inflammation and tumour development [15]. Normal pregnancy is characterized by resistance to the vasoconstrictive effects of angiotensin II [16]. In PE, there is increased sensitivity to angiotensin II as compared to that seen in normotensive pregnant women [16]. The angiotensin receptor, AT1 is a G protein-coupled receptor (GPCR) for angiotensin II, whose signalling leads to strong vasoconstriction [17]. The enhanced activation of AT_1 induces hypertension, oedema and proteinuria [18].There seems to be at least two mechanisms which operate in PE, that accelerate AT_1 signalling. These are: (1) formation of AT_1-bradykinin B_2 heterodimers [19] and (2) agonistic autoimmune antibody against AT_1 (AT_1-AA). Interestingly, increased levels of AT_1-AA are found in PE [20]. AT_1-AA may also contribute to the development of hypertension in later life, as its increased levels are observed in some women with a history of PE, even after their deliveries [21]. Stimulation of AT_1 receptor of cultured trophoblasts using IgG obtained from women with PE was found to result in the elevation of sFlt1 *in vitro* [22]. Therefore, a close association is thought to exist between accelerated AT_1 signalling and sFlt1 production. AT_1-AA is detectable in foetal cord blood in PE pregnancies, which is suggestive of its usage as a foetal-side marker for evaluating IUGR and other foetal conditions [23].

Besides it, a novel form of oxidized angiotensinogen which enhances angiotensin formation, which was found in the circulation of PE subjects [24]. Surprisingly, circulating angiotensin II and aldosterone are suppressed in PE subjects. Studies are needed to evaluate whether this oxidized form of angiotensinogen is altered before clinical disease.

Immunological markers

Placental Protein 13 (PP-13)

Placental protein 13 (PP-13) is a relatively small, 32-kDa dimer protein. It is highly expressed in the placenta. It probably has an immunobiological function at the foeto-maternal interface and in maternal vascular remodelling [25]. The levels of PP-13 in serum gradually increase in normal pregnancy, but abnormally low levels of PP-13 were detected in first trimester serum samples of women who subsequently developed preeclampsia and IUGR [26]. Furthermore, it was reported that first-trimester serum PP-13 levels may serve as a marker for early onset PE (before 34 weeks of gestation) only, but not for severe PE [27]. Combined measuring of maternal serum PP-13 and median uterine artery pulsatility index by using ultrasound early in pregnancy seems to predict severe PE [25].

Pregnancy-Associated Plasma Protein A

Pregnancy-associated plasma protein A (PAPP-A) is a large highly glycosylated protein complex which is synthesized by trophoblasts [28]. It cleaves insulin-like growth factor (IGF) binding proteins, so that free IGF can perform its biological functions. It was reported that decreased plasma levels of PAPP-A seen in first trimester were associated with PE [29]. However, PAPP-A has been demonstrated to be a more useful marker for IUGR than PE. Therefore, it is better to combine the estimation of serum PAPP-A along with uterine artery Doppler studies [30].

Metabolic marker

Visfatin

Visfatin [nicotinamide phosphoribosyl transferase (Nampt) enzyme] is an adipokine which is secreted by adipose tissue and which is involved in the biosynthesis of nicotinamide adenine dinucleotide, as it catalyzes the condensation of nicotinamide with 5-phosphoribosyl-1-pyrophosphate to yield nicotinamide mononucleotide [31]. Visfatin has been implicated in the regulation of glucose homeostasis. Its altered plasma levels are associated with various disorders, namely, type-2 diabetes mellitus, obesity, foetal growth retardation and gestational diabetes mellitus [32]. It is expressed in the placenta, foetal membranes and myometrium. It was reported that maternal plasma visfatin levels were significantly decreased in women with PE, and furthermore, its levels were correlated with severity of the disease [33]. However, another study reported that serum visfatin levels were higher in women with PE [34]. Therefore, larger scale studies are required to evaluate the role of visfatin as a potential marker for PE.

Endocrine markers

Inhibin A and Activin A

Inhibin A and Activin A are glycoproteins and members of the transforming growth factor β family. Both are largely released by the foetoplacental unit during pregnancy. Inhibin A has an important endocrine role in the negative feedback of gonadotrophins and Activin A is involved in various biological activities [35]. In normal pregnancy, concentrations of both hormones rise in third trimester, and their levels are elevated to approximately 10-fold in severe PE [36]. In PE, there is increased oxidative stress and maternal systemic inflammation. It was documented that oxidative stress stimulates activin A production and its secretion from placental explants and endothelial cells [37]. However, there are controversial reports regarding second trimester rise of inhibin A, which was not elevated as activin A in PE [38].

Recent Developments

The quest for identifying disease biomarkers that allow an accurate and early prediction of PE has intensified in the past decade. A few, selected promising biomarkers have been discussed in the preceeding section, which have potential to be used in clinical settings [Table/Fig-2]. These diagnostic tools must be able to differentiate between PE and other hypertensive disorders of pregnancy (gestational hypertension and chronic hypertension). The clinical utility of serum concentrations of angiogenic proteins in differentiating among hypertensive disorders of pregnancy has been evaluated. The diagnostic sensitivity and specificity of sFlt-1 for differentiation of PE from gestational and chronic hypertension were 84% and 95% respectively [39]. sFlt-1 measurements in plasma showed 89% diagnostic sensitivity and 90% specificity in early PE

(at <34 weeks) as compared to 55% diagnostic sensitivity and 58% specificity in late PE (at >37 weeks). Screening of urine by doing a PlGF assay, followed by a blood confirmation by checking sFlt-1/PlGF ratio, is a promising strategy. In addition, circulating concentrations of sFlt-1 were found to be increased in conjunction with decreased free PlGF in the blood, at the time of disease presentation [40]. The sFlt-1/PlGF ratio has been proposed as an index of antiangiogenic activity that reflects alterations in both biomarkers and it is also a better predictor of PE than either measure alone [40]. A recent study utilized chorionic villous samples which were taken from pregnant women at 11 weeks of gestation, to develop an mRNA profile for those who were destined to become preeclamptic [41]. The authors suggested that alterations in IL-8, MMP-9, HLA-G and chemokine (CXC motif) ligand 10 were potentially useful for assessing risk, but that altered expressions of neurokinin B and HLA-C were the most predictive. Recently, several miRNA were found to be deregulated in placentas of PE patients and they seemed to be closely associated with early pathogenesis. One such miRNA is miR-210, a novel predictive serum biomarker for preeclampsia, that can help in identifying at-risk women for monitoring and treatment [42]. Another one is miR-34a, which is involved in the pathophysiology of preeclampsia [43]. Further studies are required to develop tools for early detection and management of preeclampsia. However, question remains whether a single set of parameters are useful for diagnosing a disease entity which is as complex as PE. Therefore, it is imperative to design feasible, predictive test-set utilizing multiple biomarkers.

Name of biomarker	Plasma concentration in PE
sflt-1	increase
sEng	increase
PlGF	decrease
AT_1 AA	increase
PP-13	decrease
PAPP-A	decrease
visfatin	decrease
Inhibin A	increase
Activin A	increase

[Table/Fig-2]: Comparative analysis of the plasma concentration of potential biomarkers of PE

CONCLUSION

Recent days have witnessed a marked improvement in the management of PE patients, that has led to a decrease in mortality and morbidity caused by PE. However, challenge lies in its early diagnosis, when there are no apparent clinical signs. Therefore, an early intervention could be initiated and maternal mortality and morbidity could be reduced substantially, even in the developing countries. Till date, several promising biomarkers have been reported, which could be used to make an early diagnosis. Anti-angiogenic factors like sFlt-1, sEng and pro-angiogenic factors like VEGF, PlGF have been shown to be potential biomarkers. Especially, estimation of plasma/urine level of PlGF and sFlt-1/PlGF ratio during mid-gestation is really a promising tool. Serum AT1-AA levels could also be estimated for diagnosing PE early. Combined measuring of maternal serum PP-13 and median uterine artery pulsatility index by ultrasound early in pregnancy, seems to predict severe PE. PAPP-A has been demonstrated to be a more useful marker for IUGR than for PE. Therefore, it has been suggested that the estimation of serum PAPP-A be combined with uterine artery Doppler studies, to be used for clinical diagnostic purposes. There have been consistent reports regarding increased serum levels of activin A in PE, which have implied its utility as a diagnostic tool. But there have been controversial reports regarding serum levels of visfatin, inhibin A. However, these data came from small case studies with selected populations. Therefore, large scale multicentric prospective studies are required to confirm the specificities, sensitivities and predictive values of these promising biomarkers. It is understandable that a single biomarker cannot be used as an accurate predictive tool for diagnosing a complex disorder like PE. Therefore, analysis of a set of biomarkers is imperative for early detection of PE. However, the cost of the test, its acceptability at large and its quality control, are of paramount importance. It is hoped that extensive research being done in this arena will ultimately give rise to affordable diagnostic tools that will allow timely interventions of this complex disease process, which will help in reducing maternal morbidity and mortality significantly.

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PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Physiology, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Manisha Kar,
Associate Professor, Department of Physiology, All India Institute of Medical Sciences, Bhubaneswar,
Odisha-751019, India.
E-mail: manishasen1@gmail.com

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