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Review Article

Maternal serum biomarkers in early diagnosis of preeclampsia

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Abstract

Background: Preeclampsia (PE) is a pregnancy specific disorder, characterized by new onset of hypertension and proteinuria after 20 weeks of gestation. It is one of the leading causes of maternal and perinatal morbidity and mortality. The etiology of the disease process in not known. There is an urgent need for a 1st trimester marker for the prediction of preeclampsia. Recent studies have reported that this disease originate from abnormal placentation and maternal endothelial dysfunction. The intense research in this arena has unveiled some important serum biomarkers which play an important role in placentation. These markers include angiogenic and antiangiogenic molecules. However, these markers when they used alone are not effective for the prediction of preeclampsia, but in combination may help in predicting women who are likely to develop preeclampsia. This review summarizes the various maternal serum biomarkers available and utility in predicting preeclampsia.

Keywords: Preeclampsia, Biomarkers, Angiogenic markers, Antiangiogenic markers, Apelin

Introduction:

Preeclampsia [PE] is a pregnancy specific disorder characterized by new onset of hypertension and proteinuria after 20 weeks of gestation. [1] Globally, PE accounts for 3-5% of pregnancies and is the leading cause for maternal and perinatal morbidity and mortality. [2] In India, preeclampsia and eclampsia accounts for 24% of maternal deaths and neonatal mortality rate is approximately 43 per 1000 live births. [1] PE, a condition prior to eclampsia (Greek word "eklampsis" meaning sudden flashing), is a systemic syndrome, clearly shows the involvement of uteroplacental blood flow, vascular resistance, endothelial dysfunction, coagulation system. [1,3] The risk factors of PE include family history of hypertension, first pregnancy, chronic hypertension, diabetes mellitus, kidney disease, syndrome X, hypercoagulable state, maternal age, prolonged intervals between pregnancies, etc. [4] Symptoms of PE ranges from mild to severe. They include persistent headache, blurred vision, vomiting and abdominal pain. The complications include intrauterine growth restriction (IUGR), preterm delivery, maternal and fetal morbidity and mortality. [3]

Preeclampsia occurring at <34 weeks of gestation termed as 'early onset preeclampsia' and after 34 weeks of gestation termed as 'late onset preeclampsia'. However, in both conditions endothelial dysfunction is common and is responsible for hypertension and proteinuria. [1] Preeclampsia, occurs in two stages. Reduced placental perfusion, abnormal placentation, with improper trophoblast invasion and inadequate uterine spiral arteries remodeling occurs in stage one. Maternal inflammation, metabolic and thrombotic responses that converge to alter vascular function, results in multiorgan damage occurs in stage two. [5]

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Pathophysiology of preeclampsia:

The exact mechanism of pathophysiology of preeclampsia is unknown. However, this disease involves multiple organ systems. [6] Placenta plays an important role in pathophysiology of preeclampsia. Many studies reported that, preeclampsia is mainly due to abnormal placentation rather than foetus, it occurs only in the presence of placenta and remits after delivery. [1] In early pregnancy, maternal spiral artery remodeling starts directly after implantation of blastocyst with invasion of extravillous trophoblast

cells into decidua and formation of continuous extravillous trophoblast shell at the maternoplacental interface. [7] During this, cytotrophoblast cells invade the placental bed and transform them from low caliber resistance vessels to high caliber conduit vessels. Trophoblast invasion has specific characteristics in human placentation, limited in depth, ending in the intern third of myometrium and oriented to the spiral arteries. [6] These changes essential to allow high blood supply to uteroplacental bed and occurs at the end of 1st trimester (10-12 weeks) and ends by 18-20 weeks of gestation.[8]

During this process pseudovasculogenesis occurs, cytotrophoblasts differentiate from epithelial phenotype to endothelial phenotype and this involves direct contact with maternal blood. During preeclampsia, this transformation is incomplete. Cytotrophoblasts invades only to superficial decidua and does not reach myometrium. This abnormal cytotrophoblast invasion causes reduced uteroplacental perfusion and consequently placental insufficiency, which triggers the cascade of events leading to maternal disease. [6,9]

Pseudovasculogenesis process reduces resistance in blood vessels and increases blood supply to placenta so that it can sustain the growing fetus by providing essential nutrients and oxygen. In preeclampsia, placenta becomes hypoxic, that might trigger tissue oxidative stress, apoptosis and necrosis of placental tissue, an exaggerated inflammatory response and finally causes to endothelial dysfunction. [1] This chronic placental ischemia causes intrauterine growth restriction, and intrauterine death. [10]

Biomarkers:

The term "biomarker", a portmanteau of "biological marker", refers to a broad subcategory of medical signs-that is, objective indications of medical state observed from outside the patient-which can be measured accurately and reproducibly. In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention. [11] Due to the nonspecific signs of the disease, the preeclampsia diagnosis remains a challenge. Several studies have reported the key biomarkers associated with the pathogenic mechanism of preeclampsia, which are mainly involved in endothelial dysfunction, inflammation and placental dysfunction. [12] In this review, we discussed few promising biomarkers for the early diagnosis of preeclampsia.

Angiogenic markers:

Angiogenesis is the formation of new blood vessels, which involves the migration, growth, and differentiation of endothelial cells. The process of angiogenesis is controlled by chemical signals in the body.

In response to hypoxia, placenta produces pathogenic factors, which are involved in the endothelial dysfunction, including hypertension and proteinuria. In preeclampsia, vascular endothelial growth factor was intensively studied. Many studies suggesting that angiogenic factors play an important role and important regulators of placentation. Increased concentrations of soluble fms-like tyrosine kinase-1 (sFlt1), together with decreased concentrations of placental growth factor (PlGF) and vascular endothelial growth factor (VEGF), were the first abnormalities described. [13] List of maternal serum biomarkers were shown in table 1.

Biomarker	Plasma/serum concentration in preeclampsia
PlGF	Decrease
VEGF	Decrease
sFlt-1	Increase
sEng	Increase
RAS	Increase
PP13	Decrease
PAPP-A	Decrease
ADMA	Increase
Apelin	Decrease
NGAL	Increase

Table 1: Comparative analysis of maternal plasma/serum biomarkers for prediction of preeclampsia

Placental Growth Factor (PlGF):

PlGF, an angiogenic protein and member of vascular endothelial growth factor family and highly expressed during pregnancy. In humans, located on chromosome 14q24 and consist of seven exons spanning13.7 kb. Due to alternate splicing encoded by PlGF gene, 4 isoforms were described, PlGF-1,

PlGF-2, PlGF-3, and PlGF-4 composed of 131, 152, 203 and 224amino acids respectively. [14] Among them, PlGF-1 and PlGF-2 are the abundant isoforms. PlGF binds only to VEGFR1 (also called fms-like-tyrosine-kinase receptor/Flt-1). [15] Studies have reported that, serum and urine PlGF concentrations were significantly decreased in preeclampsia and well in advance of the disease onset. Decreased concentrations of PlGF is mostly due to a combination of reduced expression of PlGF and reduced free PlGF due its binding with sFLT1, whichis significantly increased in preeclamptic women. [16] During early pregnancy, decreased levels of PlGF is observed in women who subsequently develop preeclampsia than in normal pregnant, no difference in sFLT1, suggesting expression

of PlGF is decreased in placenta. However, at the end of pregnancy, sFLT1 and PlGF have reciprocal relationship with increasing level of sFLT1 and lower levels of PlGF, indicating that later half of pregnancy, reduced concentrations of PlGF occurs due to sequestering of PlGF by sFLT1. [17,18]

Low circulating PlGF is an indicator of abnormal placentation, data regarding the PlGF expression in placenta is conflicting. PlGF expression may be lowered due to suppression by hypoxia.

The exact mechanism of PlGF regulation is unclear, several mechanisms are associated with its regulation, especially endoplasmic reticulum stress, hypoxia -inducible factor-1α (HIF-1α) and inflammation. [19] The ratio of PlGF:sFLT1, may be one of the best predictors of preeclampsia before the disease onset. [1]

Vascular Endothelial Growth Factor (VEGF):

VEGF, also known as Vascular Permeability Factor (VPF). VEGF plays a key role in angiogenesis, vasculogenesis, and lymphangiogenesis, during embryonic and early postnatal development. In humans, located on 6p21.1 and encodes a protein which is a disulfide linked homodimer. This is a glycosylated mitogen, mainly acts on endothelial cells. [20,21] VEGF are structurally related proteins, whose members are VEGF-A, VEGF-B, VEGF-C, VEGF-D and PlGF. VEGF-A, the most abundant isoform. VEGF plays a key role in promotion of sustenance, migration and differentiation of endothelial cells and also involved in the promotion of vascular permeability. [22] VEGF- exerts its functions through two receptor tyrosine kinases, vascular endothelial growth factor receptor-1 and 2 (VEGFR1 and VEGFR2), high affinity receptor tyrosine kinases Flt-1 (VEGFR1) on placental endothelial cells and is noted to be induced by hypoxia. [23]

Several studies reported mixed results regarding the levels of VEGF, decreased [23], increased [24]. VEGF is also one of the adipose tissue- derived factor. It is known that maternal obesity and insulin resistance contributes to hypertensive disorders of pregnancy, an increased concentration of adipokines could be expected. Wolf et al., reported that increased levels of VEGF may be from adipose tissue angiogenesis. [24] Therefore, decreased concentrations of PlGF and ratio of sFLT1/PlGF during mid-gestation have been proposed as a predictive of preeclampsia.

Anti-angiogenic markers:

Soluble fms like tyrosine kinase1 (sFlt1) and soluble endoglin (sEng) are considered as antiangiogenic factors, which plays an important role in preeclampsia.

Soluble fms like tyrosine kinase 1 (sFlt1):

sFlt1is formed by alternative splicing of the FLT1 gene [1]. It contains extracellular ligand binding domain of Flt-1, but lacks the transmembrane and intracellular signaling domain. It circulates freely in the serum. sFlt -1 can binds with VEGF and PlGF with high affinity and neutralizes the biological activities of VEGF, PlGF and form VEGF-stabilized complex with extracellular domain of VEGFR2. [25] In preeclampsia, sFLT-1 is secreted at high levels. Many studies reported the relationship between elevated levels of sFlt-1 and preeclampsia. [26,27] The levels of sFlt-1 begins to rise as early as 5 weeks before the onset of the disease, supporting that, sFLT-1 is a key factor involved in the pathogenic mechanism of this disease. [28]

Soluble Endoglin (sEng):

sEng is another antiangiogenic and transmembrane glycoprotein with two splice variants, endoglin S and endoglin L. [29] Its expression is seen syncytiotrophoblast and endothelial cells. [30] Soluble endoglin is a truncated form of endoglin, it acts as an antiangiogenic factor by binding to TGFβ1 to its receptor, which finally decreases the production of nitric oxide. Throughout the normal pregnancy, the soluble endoglin levels are stable, but the levels are increased during preeclampsia. [29]

Levine RJ et al., reported that, soluble endoglin levels have been shown to be increased significantly before the onset of preeclampsia by 9-11 weeks and in term preeclampsia (>37 weeks). [31] It was implied that sFlt-1:PlGF ratio and more specifically (sFlt-1+sEng): PlGF was more strongly predictive marker for the diagnosis of preeclampsia than the individual markers.

Renin-Angiotensin System (RAS):

RAS plays a key role in the regulation of blood pressure also involved in many biological activities including vascular remodeling, inflammation and tumor development. [32,33] In addition to the angiogenic imbalance and abnormal trophoblast invasion, RAS also perturbed in preeclampsia. [34] Angiotensin II is an octapeptide, mediator of elevated blood pressure, signals vasoconstriction by binding to angiotensin II type 1 (AT1) receptor. In normal pregnancy, there is a resistance to the vasoconstrictive effects of angiotensin II. But in preeclampsia, there is an increased sensitivity to angiotensin II. [35] AT1 is the G protein-coupled receptor (GPCR) for angiotensin II and by activating many pathways such as ERK and calcineurin leads to vasoconstriction, [36] which induces increased blood pressure, edema and proteinuria. [37] There seems to be at least two mechanisms which operate in PE, that accelerate signaling (1) formation of Bradykinin B2 heterodimers and (2) agonistic autoimmune antibody against AT1 (AT1-AA). [38,39]

Placental protein 13 (PP-13):

Placental protein 13 (PP-13) is a relatively small, 32 kDa dimeric protein. First isolated form placenta, mainly form syncytiotrophoblast by Bohn et al. [40] It was a member of galactin super family, involved in placentation and remodeling of maternal arteries. It possesses a carbohydrate binding domain, to which

two proteins Annexin-II and Actin-β bind. These proteins play a key role in placental implantation and maternal vascular remodeling. [40] It probably has an immunological function at feto-maternal interface. In normally pregnancy, the concentration of PP-13 are gradually increased, but low levels of PP-13 were detected in 1st trimester serum samples of women who subsequently developed preeclampsia, particularly in cases with early onset disease. [41,42] It was reported that serum PP-13 levels in 1st trimester may serve as a marker for early onset of preeclampsia (before 34 wks of gestation) in combination with uterine artery Doppler in 1st trimester of pregnancy. [40] In a study by Romero et al., reported that maternal serum PP-13 levels in 1st trimester may serve as a marker for risk assessment for preterm preeclampsia, but not for severe preeclampsia. [43]

Pregnancy Associated Plasma Protein-A (PAPP-A):

Pregnancy Associated Plasma Protein-A (PAPP-A) is a glycoprotein complex with 1628 amino acids, which is synthesized by growing placental trophoblasts. It cleaves insulin like growth factor binding proteins (IGFBP-4), that is involved in regulating growth of the foetus. It was reported that decreased levels of plasma PAPP-A seen in 1st trimester were associated with preeclampsia. However, it was reported that PAPP-A may be a useful marker for intrauterine growth restriction than preeclampsia. [43] In a study by D'Anna et al., reported that PAPP-A levels were reduced in early onset preeclampsia while in late onset preeclampsia the levels did not differ from that of healthy control group, concluded that, 1st trimester PAPP-A is not useful in predicting late onset preeclampsia. [44]

Asymetric Dimethylarginine (ADMA):

Asymetric Dimethylarginine (ADMA) is an antiangiogenic factor that reduces the expression of vascular endothelial growth factor in endothelial cells and decreases the production of nitric oxide and lead to endothelial dysfunction. [45] During normal pregnancy, ADMA concentrations may decrease [46], but the concentrations were significantly increased in preeclampsia as it was reported by many studies [47,48]. During pregnancy, ADMA inhibits nitric oxide synthesis in rodents and produces preeclampsia signs, hypertension and proteinuria. [49,50]

Apelin:

Tatemoto et al., isolated the apelin in 1998. The formation of active apelin peptides is complex. The prepropeptide with 77 amino acids is processed into 55 amino acid intermediate and then to shorter peptides, such as apelin-36, apelin-17, apelin-13 and apelin-12. [51] In adipocytes, proprotein convertase PCSK3 or furin can hydrolyze apelin propeptide into apelin-13. Studies reported that, apelin is involved in the regulation of blood pressure. [51-53] Apelin peptides regulation in the placenta in unknown. In a animal model study by Wang C et al., reported that apelin treatment improved the preeclampsia symptoms, improved impaired eNOS/NO signaling and attenuated oxidative stress activation in reduced uterine perfusion pressure (RUPP) rats. [54] In a study by Katherine D. Bortoff et al., reported that concentrations of plasma apelin, measured at delivery were significantly low in preeclampsia compared with controls. Reduced concentrations of apelin peptides may be associated with preeclampsia. [55] In a study by Mieghem TV et al., reported that serum apelin levels 30% in pregnancies complicated by IUGR than in uncomplicated pregnancies or in preeclamptic women. In IUGR, PET, preterm and normal pregnancies placental apelin gene expression was similar and apelin staining was observed in syncytiotrophoblast and stroma. In preeclamptic and IUGR placentas, apelin staining decreased compared to normal. [56]

Neutrophil Gelatinase Associated Lipocalin (NGAL):

Neutrophil Gelatinase Associated Lipocalin (NGAL) is also known as lipocalin-2, is a secreted protein belongs to the lipocalins family. [56,57] Up-regulated expression is observed in damaged epithelial cells, inflammation, cardiovascular diseases and renal disorders. [58] Endothelial injury is a common pathophysiology involved in preeclampsia, high blood pressure and kidney injury, studies have reported that serum NGAL is increased in 2nd trimester of pregnancy in women who developed preeclampsia compared to controls. [59,60]. D'Anna R et al., reported that serum NGAL levels and their positive correlation with blood pressure, proteinuria. NGAL may be a marker for prediction of preeclampsia. [61]

Conclusions and future perspectives:

The pathophysiology of preeclampsia is complex and multiple systems are involved in patho- mechanisms. These complex interactions provide an intriguing challenge. It is important to explore the pathophysiological mechanisms involved in the early onset and late onset preeclampsia. Specifically, identification of abnormal placentation markers such as angiogenic and anti-angiogenic markers, growth factors, inflammatory markers, cytokines, antigen expression of placenta are important in this respect. Current research studies brought exciting advances in understanding the pathophysiology of preeclampsia. There have been several studies in quest for identifying new serum biomarkers for preeclampsia diagnosis. Angiogenic markers like VEGF, PlGF and antiangiogenic markers like sFlt-1, sEng have shown to be important markers for the diagnosis of preeclampsia. Especially, plas-

ma/urine levels of PlGF and sFlt-1/PlGF ratio during mid-gestation is really a promising tool. However, these data came from small case studies with selected populations. The use of this single biochemical markers for the prediction of preeclampsia are not effective. The combination of this markers along with the other predictors of preeclampsia such as maternal history, symptoms, risk factors, demographic characteristics, Doppler velocimetry will be much more useful in prediction of preeclampsia.

Recent studies suggest that metabolomics, proteomics, fetal free DNA/RNA are the some of the new techniques, which aim to generate new markers for the prediction of preeclampsia. Therefore, there is a need to conduct large scale multicentric prospective studies in order to have a better marker for the prediction of preeclampsia. Further studies are required to identify the best combination of markers that would predict preeclampsia in early.

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