INTRODUCTION
Pre-eclampsia (PE) is one of the most common complication in pregnancy. It is an important cause of maternal and perinatal morbidity and mortality. Two to eight percent of pregnancies were affected by pre-eclampsia. It is characterized by de novo hypertension and proteinuria after 20 weeks of gestation. The etiology and pathogenesis of the disease is unknown, but recent studies have revealed that placenta is the place of origin of this disorder and widespread maternal endothelial dysfunction is the characteristic feature of the disease. Some biochemical molecules are identified recently which are involved in the pathogenesis of the disease, which may help in early identification of patients at risk and help in providing proper prenatal care. Several promising biomarkers have been proposed, alone or in combination. Maternal serum concentrations of these biomarkers either increase or decrease in pre-eclampsia during gestation. This review focuses on the various biomarkers available and their utility in prediction and diagnosis of pre-eclampsia.

PATHOGENESIS
The etiology of preeclampsia is unknown. Evidence suggests that the presence of a placenta but not necessarily a fetus is required for the development of this disorder. The pathogenesis of preeclampsia is not completely clear. It is a multifactorial disease. Several mechanisms have been implicated in the pathogenesis of preeclampsia, including endothelial dysfunction, inflammatory pathway, oxidative stress and the renin–angiotensin system. It has been considered as a two stage disease in which abnormal placental development precedes endothelial dysfunction. In normal placental development,
the cytotrophoblasts invade the maternal spiral arterioles and transform them from small caliber resistance vessels to high caliber conduit vessels. The beginning of this initial event has occurred around 10-12 week and completed by 18 to 20 week of gestation. During this vascular invasion the cytotrophoblasts differentiate from epithelial phenotype to an endothelial phenotype, a process known as pseudo vasculogenesis. 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. During pre-eclampsia, the invasive cytotrophoblasts fail to transform epithelial phenotype into endothelial phenotype along with shallow invasion of the spiral arteriole which leads to defective uteroplacental circulation and worsening placental perfusion causing placental ischemia and hypoxia. All these directly or indirectly damage endothelial cell function. Generalized endothelial dysfunction with systemic inflammatory response is thought to be the final common pathway that leads to the maternal signs of preeclampsia with de novo hypertension and proteinuria in the second half of pregnancy.

As preeclampsia is very common in pregnancy and carries a high maternal and perinatal morbidity and mortality, a number of methods are tried to predict the development of preeclampsia so that we can identify these women early and appropriate measures can be taken for safe pregnancy outcomes.

Prediction is basically depend on clinical tests, such as blood pressure measurement during the second trimester or 24-hour ambulatory blood pressure monitoring, angiotensin infusion test, roll over test and some more, but these lack sensitivity and specificity. Many biomarkers have been evaluated for diagnosis of PE which could also help in the accurate prediction of the PE in the first trimester itself. This review thus focuses on the available biomarkers and their utility. Some of them are described below.

**Angiogenic factors**

Circulating factors that regulate blood vessel formation and health, referred to as angiogenic factors. Some novel soluble angiogenic factors are identified that are related to the pathogenesis of the disease. Angiogenic factors are thought to be important in the regulation of placental vascular development. These factors include circulating antiangiogenic proteins such as soluble fms-like tyrosine kinase- 1(sflt-1) and soluble endoglin (sEng) and proangiogenic protein such as placental growth factor (PIGF) and vascular endothelial growth factor (VEGF). Their receptors, fms-like tyrosine kinase or Flt-1, also known as vascular endothelial growth factor receptor-1 (VEGFR-1), VEGFR-2, Tie-1, and Tie-2, are essential for normal placental vascular development. A strong association between altered circulating angiogenic factors and preeclampsia has been demonstrated by several studies. The higher relative concentration of antiangiogenic factors are believed to trigger vascular endothelial cell injury in the liver, kidney, brain as well as in the placenta.

**Endoglin**

Endoglin, an antiangiogenic protein has been implicated in the pathogenesis of PE. It is a 180 kDa, homodimeric, type I membrane glycoprotein located on cell surfaces. It is also commonly referred to as CD105, END, FLJ41744, HHT1, ORW and ORW1. It is a part of the transforming growth factor (TGF) beta receptor complex expressed at high levels on vascular endothelial cells and functions as an antiangiogenic factor by binding transforming growth factor b-1 (TGFb-1) and TGFb-3 proteins which are important for angiogenesis. Several studies have demonstrated that endoglin is expressed in human first trimester decidua cells and is highly upregulated in the syncytiotrophoblast of women with preeclampsia and concentration of its soluble form is increased into the circulation of preeclamptic women. This soluble form of endoglin (sEng) is produced by the proteolytic cleaving action of MMP-14 (Matrix metalloproteinase) in extracellular domain. It acts by antagonizing an angiogenic and vasodilator molecule known as transforming growth factor beta-1, which is important in angiogenesis and also maintain the health of the blood vessels. Due to this, cell lining the blood vessels begin to sicken and die, blood pressure increases and blood vessels leak protein into the tissues and urine. It is an important protein for tumor growth, survival and metastasis of cancer cells to other locations in the body.

In human pregnancy, alterations in serum sEng antedate clinical symptoms of preeclampsia by several months before the onset of disease. Because high levels of serum sEng are released into the human circulation prior to the clinical manifestations of
pre-eclampsia, this glycoprotein has been proposed recently as a serum diagnostic biomarker for pre-eclampsia. Levine et al 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:PIGF ratio and more specifically (sFlt-1+sEng): PlGF is a stronger predictor of pre-eclampsia in comparison to individual markers.

**Vascular Endothelial Growth Factor (VEGF), Placental Growth Factor (PIGF)**

Among the various angiogenic factors expressed by the placenta, VEGF and PIGF play a very important role. VEGFs are a family of structurally related dimeric proteins whose members include VEGF-A, VEGF-B, VEGF-C, VEGF-D and PIGF. VEGF play an important role to promote sustenance, migration and differentiation of endothelial cells and also maintain the vascular permeability. VEGF interact with VEGFR-2 and VEGFR-1 on the placental endothelial cells. Several studies have reported that serum concentration of VEGF is reduced in pre-eclamptic patients. That is why the activity of sFlt-1 (a soluble form of VEGF receptor-1 or sVEGFR-1) is upregulated in pre-eclamptic conditions. Increased levels of free serum sFlt1 bind with both VEGF and PIGF, thereby neutralizing them, and subsequently their levels in circulation reduced. There is also decreased production of VEGF by circulating T and natural killer cells in pre-eclampsia, it also play a role in endothelial dysfunction which is characteristic of the maternal syndrome of the disease. Although for prediction of PE, VEGF has been studied as a promising marker but it could not be detected by many available ELISA kit because its circulatory levels are very low. To overcome this limitation, highly sensitive ELISA kits can be used.

One of the most important members of VEGF family is placental growth factor (PIGF). It also has an important role in angiogenesis and placental vasculature. Placental trophoblasts are the major source of PIGF. PIGF-1, PIGF-2, PIGF-3, and PIGF-4 are the different isoforms of the PIGF. PIGF binds only to VEGFR-1. In women who are destined to develop PE, the splice variant of VEGFR-1, the sFlt-1, readily neutralizes the PI GF, hence its level in the serum reduces. Various studies have demonstrated that maternal serum levels of PIGF are lower in both early and late onset PE. Various studies suggest, the best method for prediction of PE is PIGF: sFlt-1 ratio.

**Soluble FMS-like tyrosine kinase -1 (SFLT-1)**

It 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 PIGF. When compared with control subjects, the women who develop PE, a significant rise in serum levels of sFlt-1 was shown by several studies. The sFlt-1 specific ELISA kits are used for estimation of its serum levels. Baumann et al reported the predictive role of sFlt1 and sEng in PE. Levine et al also found that higher levels of serum sFlt-1 are predictive of PE. However, some studies showed the lower specificity and poor predictive value of sFlt-1 in the early stages of pregnancy.

**Inhibin-A and Activin-A**

These glycoproteins are produced by the fetoplacental unit. In patients who subsequently developed PE, higher serum levels of these glycoproteins are found in their first trimester. Hence, these can be used in prediction of PE.

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

It is 1628 amino acid peptide. It is mainly produced by the trophoblastic cells. It has a role in cleavage of insulin like growth factor binding proteins. It has a role in regulation of fetal growth. Some studies have shown that plasma levels of PAPP-A has decreased in all trimesters of pregnancy, some other studies indicate that the levels of PAPP-A were significantly reduced in the early onset PE while in case of late onset PE the levels did not differ from the control group. Hence, PAPP-A is not useful in predicting late onset PE and larger trials are required to confirm these preliminary predictions.

**Neutrophil Gelatinase-Associated Lipocalin (NGAL)**

Neutrophil gelatinase-associated lipocalin (NGAL), is a 25 kDa protein and belongs to the lipocalins family. It is also known as lipocalin-2, siderocalin, uterocalin and 24p3. 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.\textsuperscript{50} NGAL can be detected in urine within two hours of kidney damage, hence it is considered as the best and the earliest markers of acute kidney damage.\textsuperscript{50,51} In some recent studies serum level of NGAL was found to be increased at the end of second trimester in women who subsequently developed PE compared to control group.\textsuperscript{47,49,52} A positive correlation of serum NGAL was found with the systolic and diastolic blood pressure and with proteinuria.\textsuperscript{38,47,49} Hence serum NGAL can be used as a reliable biomarker for early prediction of pre-eclampsia.

**Placental Protein 13 (PP-13)**

Placental protein 13 (PP13) is a 32 kDa dimeric protein. It was first isolated in 1983 from the syncytiotrophoblast of the placenta by Bohn et al\textsuperscript{54,55}. It was identified as a member of the galectin superfamily, which has an important role in placental implantation and remodelling of maternal arteries.\textsuperscript{38,56} To perform this function PP13 has a carbohydrate binding domain, to which two proteins Annexin-II and Actin-beta bind. In normal pregnancy, PP13 levels are gradually increasing while abnormally low levels of PP13 were found in first trimester of women who subsequently developed PE, compared with controls.\textsuperscript{53,56,57} Hence, it can be used as serum biomarker for prediction of PE.\textsuperscript{53}

Nicolaides et al demonstrated that the combination of serum PP13 levels and uterine artery pulsating index measured by Doppler ultrasonography have a good prediction rate to identify the patients having the risk of developing pre-eclampsia in the first trimester.\textsuperscript{56} PP13 specific ELISA kits are used to measure the serum PP13 levels with good sensitivity and specificity.\textsuperscript{55,56} Hence, by using serum PP13 alone or in combination with Doppler studies, early identification of high risk patients can be done and we have a good opportunity for implementation of treatment strategies.\textsuperscript{53,56-58}

**Pentraxin-Related Protein 3 (PTX3)**

PTX3 is TNF-inducible gene 14 protein (TSG-14)). This protein is encoded by the PTX3 gene in humans.\textsuperscript{59} Maternal plasma levels of PTX3 was found to be elevated in preeclamptic women in comparison to control group, supporting the excessive maternal inflammatory response to pregnancy as one of the etiology of PE.\textsuperscript{60}

**P-selectin**

P-selectin is a protein encoded by the SELP gene in humans.\textsuperscript{61} Platelet activation in PE is reflected by elevated levels of platelets exposing P-selectin. In plasma, a non-cell bound (soluble) form of P-selectin is present. Elevated levels of this soluble form have been reported in PE.

**Other tests**

Laboratory tests for oxidative response, i.e. malondialdehyde along with antioxidants have been assessed, including assays for uric acid, urinary kallikrein, fibronectin and cytokines but till date no test was found relevant.\textsuperscript{62-66} Because no single marker effectively predicts the risk of PE, hence in clinical practice, the current trend is to test a combination of markers. Larger studies are required to label a single molecule as a biomarker for the early prediction of PE.

**CONCLUSION**

Our understanding about etiology and pathogenesis of PE has been improved a lot in last decade. Several studies have been done and a lot of biomarkers were studied for prediction and diagnosis of PE such as anti-angiogenic factors like serum soluble endoglin, sFlt-1, sEng and pro-angiogenic factors like VEGF, PIGF. 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 many other issues. Hence further studies are required with larger population and with more precise and advanced techniques.

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