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DISEASES

Narrative review

Prediction of the preeclampsia: a view of biochemical markers

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Abstract

Preeclampsia is a diverse, multiorgan group of related disease processes that occurs in up to 5%-8% of pregnancies after 20 weeks' gestation and it is one of the leading causes of maternal and fetal morbidity and mortality. Many molecular mechanisms are contributed to the pathogenesis of preeclampsia. Although it is unknown whether the mechanisms act independently or have synergistic effects. This review describes review of primary papers investigating blood based biomarker such as PAP-A, Inhibin A, sFlt1, and PP13 in general and first trimester biochemical markers and combinations of them specifically for preeclampsia.

Keywords

Preeclampsia; Biochemical markers; Pregnancy-associated placental protein A

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Disclosure
The authors declare that they have no financial competing interests

Introduction

Hypertensive disorders of pregnancy are important causes of maternal and fetal mortality and morbidity. Along with infection and hemorrhage, it stands among the three most common causes of maternal mortality. The reported rate of hypertension with pregnancy is 5-10% of all pregnancies [1]. Major risk factors for preeclampsia (PE) include nulliparity, multifetal gestations, previous history of preeclampsia, obesity, diabetes mellitus, vascular and connective tissue disorders like systemic lupus erythematosus and antiphospolipid antibodies, age >35 years at first pregnancy, smoking. Preeclampsia is considered a disease of nulliparous women, as it is twice as common in primigravidas as it is in women who have previously given birth [2]. PE is characterized by a complex pathophysiology and heterogeneous clinical and laboratory findings. The relation between these risk factors and preeclampsia is poorly understood. Most theories on the etiology of preeclampsia suggest that the disease is a cascade triggered by combination of abnormal maternal inflammatory response, endothelial cell activation/damage with deranged hemodynamic milieu, and deranged immunity [3,4].

The current theory of the pathogenesis of PE as reviewed by Christopher Redman and Ian Sargent is thought to occur as a 2-stage process with poor placentation in the first half of pregnancy resulting in the maternal response in the second half of pregnancy [5,6].

Increasing evidence suggests that an excessive maternal systemic inflammatory response to pregnancy with activation of both the innate and adaptive arms of the immune system is involved in the pathogenesis of the disease [7]. After conception, regulatory T cells, interacting with indoleamine 2,3-dioxygenase, together with decidual NK cell recognition of fetal HLA-C on extravillous trophoblast may facilitate placental growth by immunoregulation. Complete failure of this mechanism would cause miscarriage, while partial failure would cause poor placentation and dysfunctional uteroplacental perfusion [8]. Bayram et al. found that the patient with severe preeclampsia have higher values of IL-6 and IL-1β when compared to the normotensive ones [9]. Increased level of IL-6 and TNF-α may play a role of triggering factors for preeclampsia. Uterine NK cells, the major lymphocytes present in the decidua during pregnancy, have the ability to produce and be regulated by IL-10 [10]. Kalkunte et al. showed that reduced production of IL-10 may contribute to poor placentation and induction of vasoactive antiangiogenic factors. Curiously, evaluation of placental tissue and serum samples from PE has suggested reduced IL-10 production [11].

Currently, no definitive treatment or effective prophylaxis for preeclampsia is available. Delivery and consequent removal of the placenta is the "treatment", often with a premature baby as the result.

The National High Blood Pressure Education Program of the NHLBI classifies hypertensive disorders of pregnancy into following categories: gestational hypertension, chronic hypertension, preeclampsia, and preeclampsia superimposed on preexisting hypertension [5].

According to the International Society for the Study of Hypertension in Pregnancy (ISSHP), PE can be defined as de novo hypertension occurring after 20 weeks of pregnancy together with proteinurea. Hypertension is defined as a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg measured at two occasions with at least 4 h in between. Proteinurea is defined as ≥ 300 mg per day in at least 2 random urine specimens that were collected at least ≥ 4 hours apart (but within a 7-day interval) or 0.3 g in a 24-hour period [6].

In the recent years, facilities for the researches have been made more widely available. Genomics, proteomics and metabolomics modalities are popular and possible to evaluate. In search of the etiology of PE, several new pathways and factors have been described by using these techniques. So that the researchers were able to work on the etiology of PE may affect the new pathways and markers. Aim of these researches is to describe the etiological factors measurable in the maternal blood and their evaluation as biochemical markers for prediction and diagnosis of PE. The main of these markers are about endothelial dysfunction, metabolic status, oxidative stress, placenta-derived factors, hemolysis

and inflammatory markers. Also serum/plasma markers for renal dysfunction are important about the etiology of PE.

The ultimate goal of a preeclampsia biomarkers would be that appropriate improving maternal health situation and detection of the conditions which affect development of the fetus. As a result of definition these markers could possibly improve our understanding of the pathophysiology in preeclampsia. By the way identification of new therapeutic targets, as many of the markers probably contribute to the pathophysiology can help to find appropriate treatment modalities. Also understanding the exact pathophysiology of PE may allow clinician to prevent disease before the symptoms occur.

Few biochemical markers have been proven specific and sensitive as single markers to predict and/or diagnose PE. Beside these biochemical markers clinical measurements such as Doppler ultrasound is important for estimation of the PE. In this review we describe biochemical markers suggested for both prediction and diagnosis of PE.

Biochemical markers

Pregnancy-associated placental protein A

Pregnancy-associated placental protein A (PAPP-A) is a large, highly glycosylated protein synthesized in the placenta by the developing trophoblast. The study of PAPP-A as a biochemical marker in pregnancy has been pursued for almost 30 years [12]. PAPP-A has been used in combination with β-human chorionic gonadotropin (β-hCG) and nuchal translucency thickness. Aim of using these three markers is detection of Down syndrome at 11 + 0 to 13 + 6 weeks of gestation [13].

PAPP-A has been evaluated as a predictive and diagnostic biochemical marker for PE. But the screening performance is extremely low when used as a single biochemical marker and the predictive rate is only about 10-20 % [14]. Several studies denoted low first or second trimester serum PAPP-A value is predictive and diagnostic biochemical marker of preeclampsia [14-23]. First trimester serum PAPP-A is low also prior to other pregnancy complications, such as in pregnancies developing fetal growth restriction, spontaneous miscarriage placental abruption, preterm birth and stillbirth and gestational diabetes [15,16,20,23]. Because of these other complication of the pregnancy usefulness of PAPP-A as a preeclampsia biomarker is restricted (Table I). D'Anna et al. showed that PAPP-A is not a predictive marker for patients with onset of preeclampsia after 34 week of the gestation [21]. When the combina-

Reference	Pz (n.)	Gestational week	Results
Ong [15]	5,584	10-14	Low PAPP-A prediction of PE, PIH, miscarriage, FGR
Smith [16]	8,839	8-14	Low PAPP-A prediction of PE; FGR, premature birth, stillbirth
Bersinger [17]	118	17, 25, 33	Low PAPP-A at week 17 predicts PE also FGR
Dugoff [20]	33,395	10-14	Low PAPP-A predicts adverse pregnancy outcome, included PE and PIH, fetal loss, SGA, placental abruption, preterm premature rupture of membranes. Low sensitivity and PPV
D'Anna [21]	2,432	9-11	Low PAPP-A not predictive of PE

Table I. Biochemical prediction of Pregnancy-associated Protein A (PAPP-A) as marker of preeclampsia

FGR = fetal growth restriction; PE = preeclampsia; PIH = Pregnancy induced hypertension; PPV = positive predictive value; SGA = small for gestational age | siRNA (small interfering RNA)

tion of PAPP-A with Doppler ultrasound is used, PAPP-A became a powerful predictive biochemical marker of PE with prediction rates of 70 % at false positive rates of 5% [18]. Nonetheless the sensitivity of PAPP-A is still too low to provide useful screening biomarker for preeclampsia. As the result of these PAPP-A might be a better marker for fetal growth restriction than preeclampsia [22].

Inhibin A and activin A

Inhibin A and activin A are glycoprotein hormones and members of the TGF (transforming growth factor)-β family. The placenta is the primary source of inhibin A and activin A during pregnancy. The concentrations of these circulating proteins increase with the last trimester of uncomplicated pregnancies. Muttukrishna et al. found that maternal serum concentrations of inhibin A and activin A are more elevated in women with preeclampsia as compared to normotensive control women [23]. Some authors have confirmed that elevated inhibin A/activin A levels in first trimester [24,25] or in second trimester [26-30] could predict preeclampsia (Table II). Spence et al. found that elevated inhibin A and activin A levels combined with uterine artery doppler measurement become a more accurate predictive values in second trimester [30].

Placental protein 13

Placental protein 13 (PP13) is a member of the galectin family and is a 32-kDa dimer protein that is produced by the trophoblast cells in placenta [31,32]. The real function of PP13 is still not clearly understood, but it is involved in placental implantation and maternal vascular remodeling [31]. Several studies have shown lowered serum level of PP13 in the first trimester in pregnancies that subsequently developed PE (Table III). As a first trimester screening marker for PE, PP13 shows different prediction rates in different studies. In two different cohort studies, PP13 levels were determined at 11 + 0 to 13 + 6 weeks of gestation [33,34]. At false positive rates of 5% and 10% they found detection rates of 37.5% and 69% respectively, using PP13 as a single biochemical marker. It seems that a combination of PP13 and uterine artery Doppler PI is a better prediction test for preeclampsia than either test alone, either if performed simultaneously in gestational week 11-14. When combining serum screening with Doppler ultrasound pulsatility index (PI), the prediction rate increased to 71% at a false positive rate of 10% [34]. Nicolaides et al. found that PP13 is a biochemical marker for early onset PE in gestational week 11-14 [35]. At a false positive rate of 10%, PP13 showed a prediction rate of 80% as a single biochemical marker. In combination with Doppler ultrasound PI measurements, the prediction rate increased to 90% [35]. PP13 has also been used in combination with PAPP-A. Stamatopoulou et al. studied PP13 and PAPP-A at 11 + 0 to 13 + 6 weeks of gestation in hypertensive pregnancies and pregnancies complicated by SGA. They found that PAPP-A was significantly lower in SGA and in hypertensive disorders but interestingly, the levels of PP13 did not differ between the cases and the controls [36].

Reference	Pz (n.)	Gestational week	Results
Roes [25]	36	6-15	Elevated inhibin-A predicts PE. Sensitivity (32%)
Diesch [26]	15	19-24	Second trimester elevated activin A in a high-risk population predicts PE
Zwahlen [27]	52	11-14	Increased activin A and inhibin A predicts PE
Muttukrishna [28]	1,596	15-19	Elevated inhibin A and activin A predicted PE. Activin A is more predictive than inhibin A
Spencer [30]	24	22-25	UA Doppler in addition to elevated activin A and inhibin A gave better prediction for PE than either markers alone

Table II. Biochemical prediction of Inhibin A and Activin A as marker of preeclampsia

PE = preclampsia, UA = uterine artery

Reference	Pz (n.)	Gestational week	Results
Akolekar [33]	48	11-14	PP13 is predictive for subsequent development of early PE
Khalil [34]	42	11-14	Combination of first-trimester PP13, uterine artery PI analysis is promising for the prediction of PE
Nicolaides [35]	423	11-14	Low PP13 predicts PE. Uterine artery PI improves detection rate of early onset PE: 90% detection rate
Stamatopoulou [36]	452	11-14	PP13 did not differ between the cases and the controls

Table III. Biochemical prediction of Placental protein 13 as marker of preeclampsia

PE = preeclampsia; PI = pulsatility index; PP13 = placental protein 13; UA = uterine artery

Vascular endothelial growth factor

Circulating free vascular endothelial growth factor (VEGF) is very low in pregnancy. The main problem to use VEGF to demonstrate as a marker is hard to detect it with the Elisa kits. Polliotti et al. demonstrated that a low VEGF was predictive of subsequent preeclampsia [37]. Levine et al. found that free VEGF was lower in women developing preeclampsia as compared to women remaining normotensive only 5 weeks before disease development [38].

Placental growth factor

Placental growth factor (PlGF) is a member of the VEGF family which secreted by trophoblast cells of placenta. PIGF has a proangiogenic function at the site of the placenta. Preeclampsia occurs as the result of impaired placentation. Subsequent ischemic situation occurs because of an increased secretion of antiangiogenic factors such as soluble Fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) in the maternal circulation. By the way this situation leads to a course of antagonizing the angiogenic factors such as PlGF. Some studies have found that low PlGF is a good predictive factor in first trimester for preeclampsia [39-43], while other studies showed no predictive value for PlGF in first trimester [44-47]. Most studies have found that low PlGF in the second trimester predicts later occurrence of preeclampsia [47-54]. The ratio of the PlGF/sFlt-1 is well described and this new value has shown as an appropriate biochemical marker for prediction of PE [55]. Several studies have shown the predictive power of PlGF/sFlt-1 ratio from the second trimester (Table IV).

Reference	Pz (n.)	Gestational week	Results
Tidwell [39]	39	5-15 16-20 26-30	Low PlGF week all trimesters predict severe PE. Low PlGF week 5-15 (but not later in pregnancy) predicts mild PE
Thadhani [40]	80	7-12	1st trimester low PlGF predicts PE, not SGA
Taylor [44]	1,496	Full term	PlGfF <14 th week: does not predict PE/SGA. Low PlGF predicts PE + SGA from week 15-19. Low PlGF predicts PE as well as PE + SGA from week 21-25
Tjoa [47]	72	11-21	PlGF week 14-17 does not predict PE. Low PlGF week 17-21 predicts PE
Espinazo [52]	3,348	22-26	2nd trimester low PLGF predicts PE (in women with abnormal UA Doppler)

Table IV. Biochemical prediction of Placental growth factor as marker of preeclampsia

PE = preeclampsia; PlGF = placental growth factor; SGA = small for gestational age; UA = uterine artery

De Vivo et al. found that the prediction rate of PlGF/sFlt-1 ratio is about 89% for PE [56]. As a single biochemical marker, PlGF has been shown to predict 53.5% of early onset PE at a false positive rate of 5% and 65% at a false positive rate of 10% in late first trimester [55]. PIGF value is also low with pregnancy complications associated with a dysfunctional placenta (SGA, preeclampsia or both) [44]. Several studies have found that low PlGF concentrations in first trimester are also predictive factor for SGA, even in the absence of preeclampsia [40,42,47].

Soluble Fms-like tyrosine kinase-1

Most studies about the role sFlt1 in PE evaluation have shown that elevated second trimester sFlt1 predicts preeclampsia (Table V) [29,38,41,57-59]. sFlt1 is a better predictor for the early-onset type of preeclampsia, which has worse pregnancy outcomes when compare with the late onset type of preeclampsia.

As for sFlt1 measured in first trimester blood samples, there are discrepant findings. Levine et al. found elevated levels of circulating sFlt1 5 weeks prior to clinical preeclampsia, but detected no association with sFlt1 in first trimester [38]. In contrast, a large nested case-control study within a cohort of nearly 30,000 pregnant women in Norway showed that low sFlt1 in first trimester predicted early onset preeclampsia [41]. Kusanovic et al. also had confirmed that low sFlt1 in first trimester predicting preterm (as well as term) preeclampsia [43] and Erez et al. [42]. Although after adjusting for BMI, age and nulliparity, this significance had disappeared.

Soluble endoglin

Soluble endoglin (s-Eng) has been found elevated approximately 2-3 months before clinical presentation of the PE [60]. When, s-Eng used as first trimester screening marker, it shows conflicting results [42]. The prediction rate for early onset PE was 77.8% at a false positive rate of 5% (61) when s-Eng is used with Doppler ultrasound and PlGF.

Cystatin C

Cystatin C is a protease inhibitor and it has widely usage area by clinicians as a sensitive marker for renal function and for estimation of glomerular filtration rate. The maternal plasma level of cystatin C

Reference	Pz (n.)	Gestational week	Results
Sibia [29]	104	12-20 24-28	sFlt1 at week 16 does not predict PE. End of second trimester sFlt1 and sFlt1/PlGF ratio predicts preterm delivered PE
Levine [38]	120	>13	Elevated sFlt1in second trimester predicts PE and is elevated as compared to controls 5 weeks prior to clinical PE
Vatten [41]	344	4-12 19-27	Low sFlt1 in first trimester predicts early-onset PE. High sFlt1 second trimester predicts early-onset PE. High increase in sFlt1 from first to second trimester strongly predicts PE
Moore [58]	94	22-26 27-30 31-35	Second trimester elevated sFlt1 predicts early-onset PE. Elevated sFlt1 predictive for term PE only late in pregnancy (week 31–35). Elevated late sFlt1 predicts all forms of PE
Stephan [59]	8	22	Elevated sFlt1 prior to PE in high-risk population

Table V. Biochemical prediction of soluble Fms-like tyrosine kinase-1 (sFtl1) as marker of preeclampsia

PE = preeclampsia; PIGF =placental growth factor

is increased in women with PE and authors had demonstrated that the level of cystatin C is a reliable diagnostic marker for PE [62,63]. Recently cystatin C has been suggested as a predictive first trimester marker for PE [64]. However, the low screening rate of the study, cystatin C is probably not clinically useful as a single marker [65]. Thilaganathan et al. showed that cystatin C could be useful in combination with other predictive markers [65].

PTX3

Pentraxin 3 (PTX3) is a secreted protein as part of an inflammatory immune response and is increased as an acute phase protein molecule like acute phase reactant [66]. Both with manifestations of PE as well before clinical symptoms, there is an increased secretion of PTX3 in the maternal circulation [67,68].

P-selectin

P-selectin is a cell adhesion molecule, and plays a role in endothelial dysfunction. Placental ischemia and endothelial dysfunction are main components in the context of preeclampsia and as a result of these P-selectin levels found in high serum concentrations [69]. This situation mainly detectable in the first trimester of pregnancy [67,68].

IGFBP-1 and 3

Both insulin-like growth factor binding proteins are the focus of the new researches. Both, in early- and late- onset preeclampsia, IGFBP-1 is decreased in the first trimester. Such changes are detected by secretion of when we investigate the levels of the IGFBP-3, it elevates only in late-onset preeclampsia. In both cases, there is no correlation to a disturbed trophoblast invasion [70,71].

Adiponectin

Nanda et al. found that adiponectin levels increase in early PE but not in late PE and that there is no significant association between adiponectin and PAPP-A or uterine artery PI. Serum adiponectin did not improve the performance of screening for PE provided by a combination of the maternal factors, uterine artery PI and serum PAPP-A [72].

Discussion and conclusions

Preeclampsia is associated with significant morbidity and mortality for both mother and fetus. Most important objective for this disorder should be having chance of early diagnosis and develop an approach using biomarkers to detect, as early as possible, women at risk of developing preeclampsia with a greater performance than conventional methods. This situation offers a targeted manner, prophylactic interventions, specific surveillance and closer management. Although many different potential markers exist for preeclampsia, the reliability of these markers in predicting preeclampsia has been inconsistent between different studies. When we reviewed the studies about preeclampsia none of the biochemical markers has shown to be universally acceptable and reliable potentially predictive value for preeclampsia.

The appropriate biochemical marker for PE should give idea in the pathogenesis and be specific for the status of the disease. They also appear at the beginning of pregnancy or before the clinical manifestations of the disease with high sensitivity and specificity for early diagnosis and enough time for chance for the treatment. These tests must be easy and cheap to measure in maternal blood universal usage. Also they must correlate with the severity of the disease for exact diagnosis and using required treatment modalities. Cnossen et al. reviewed tests for predicting preeclampsia, performing a meta-analysis of 219 studies. At the end of the study, they found the accuracy of 27 tests for predicting preeclampsia, among these

were 10 blood based biochemistry tests [73]. No test had a high level of both sensitivity and specificity greater than 90%.

Several authors have studied that combination of markers improves test accuracy in predicting preeclampsia. Because of the complex structure of the PE, a combination of independent biomarkers, each reflecting a different part of the pathophysiology of the disease, should increase the likelihood to derive appropriate predictive methods [74,75]. Screening for Downs's syndrome in the first trimester is a good example where a combination of ultrasound scanning and biochemical markers are used. For instance, the combination of uterine artery Doppler pathological findings with altered biochemical markers in second trimester have shown better than use of the Doppler test or the factors alone for prediction of the PE [59,76-78] . Stepan et al. showed that combination of abnormal Doppler findings with sFlt1 increased the sensitivity of Doppler alone for preterm delivery from 67% to 83% and the specificity from 76% to 89% [59]. Crispi et al. found 90% sensitivity and 95% specificity for identifying early onset preeclampsia when using the combination of second trimester serum PlGF and uterine artery mean pulsatility index [77]. Stepan et al. also recently showed that using the combination of sFlt1 and sEng in second trimester was able to predict early-onset preeclampsia in women with abnormal uterine artery Doppler findings with 100% sensitivity and 93% specificity [77].

New technologic developments that may aid the search of new screening biological markers include gel-free proteomics with a mass-spectrometry approach that identify global protein changes in plasma. Blankley et al. showed elevation of several circulating proteins in pregnancies with preeclampsia as compared to normal pregnancies, including PAPP-A, endoglin and pregnancy-specific-beta-1-glycoprotein 1. This situation is currently under investigation whether this proteomics approach can identify global protein differences also prior to disease [79]. Metabolomic products have also been suggested as a investigation modality for identifying new biomarkers for preeclampsia. Kenny et al. identified eight metabolomic products that with high sensitivity and specificity differentiated pregnancies with preeclampsia from control pregnancy blood [80].

Current researchers focus on the prediction of onset of preeclampsia so as to allow early management and improve the morbidity and mortality associated with this disease. The appropriate biochemical marker for PE should give idea in the pathogenesis and be specific for the status of the disease. As preeclampsia has a complex pathophysiology, the pursuit of a single biomarker that will predict all sorts of clinical situation of the disease, may prove difficult. Recent studies carried out in the direction of a combination of the biochemical and other markers. However, not yet been found fully appropriate marker. More extensive and appropriate studies should be done about it.

Future directions for research

Future studies should be undertaken focusing on finding new markers, or a combination of them, that will early predict the onset of preeclampsia.

The review in brief	
Clinical question	Describe biochemical markers suggested for both prediction and diagnosis of PE
Type of review	Narrative
Conclusions and limitations	Although many different potential markers exist for preeclampsia, the reliability of these markers in predicting preeclampsia has been inconsistent between different studies. Recent studies carried out in the direction of a combination of the biochemical and other markers

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