



Biomarkers and genetic factors for early prediction of pre-eclampsia

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Pre-eclampsia is known to cause considerable maternal morbidity and mortality. Thus, many studies have examined the etiopathogenesis of pre-eclampsia. While many pathophysiological factors related to pre-eclampsia have been identified, the precise etiopathogenesis of pre-eclampsia remains unclear. Numerous studies have identified factors for the early prediction for pre-eclampsia to lead to preparation and closer observation on pre-eclampsia when it occurs. This article reviews on current studies of biomarkers and genetic factors related to pre-eclampsia, which may be important for developing strategies for early prediction of pre-eclampsia.

Key words: Pre-eclampsia, Biomarkers, Genes, Cell-free nucleic acids, Early detection of disease.

Introduction

Pre-eclampsia (PE) is a disorder that occurs in multiple systems during pregnancy and it causes considerable maternal morbidity and mortality. PE is a serious complication characterized by high blood pressure and proteinuria, and it can lead to multiple organ damages. It typically develops after 20 weeks of pregnancy, but it can occur at any time during labor, or even up to 6 weeks after delivery. PE can become severe very quickly or progress slowly. Left untreated, PE can lead to dangerous—or even fatal—complications for both the mother and fetus, such as HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome and eclampsia [1–3].

The pathogenesis of PE remains unclear despite extensive research over the past few decades. However, multiple factors related to PE including maternal and fetal/placental factors have been identified. In PE, invasion of the maternal uterine spiral arteries into the placental trophoblast is inadequate. This is one

of the main causes of poor placental perfusion and leads to placental and fetal hypoxia. Hypoxia is a potent stimulus for release of the numerous factors into the maternal circulation that may affect endothelial function of maternal system, and this can be reason of hypertension and other signs of the disease.

Currently, PE is thought to result from defective spiral artery remodeling, leading to cellular ischemia in the placenta and resulting widespread endothelial dysfunction of maternal multiorgan systems. Exact etiology underlying the cellular and molecular mechanisms of PE is still elusive. But recent observations support that altered expression of multiple factors is responsible for the clinical manifestation of the disease [4].

PE is divided to 2 types; early-onset and late-onset PE. Early-onset PE typically requires early delivery (before 34 weeks' gestation) as intrauterine growth retardation, unusual uterine and umbilical artery Doppler waveforms, and negative effects on the maternal and neonatal sides are common. Late-onset PE is commonly related to mild maternal disease and a low rate of

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fetal involvement, with delivery typically at or after 34 weeks of gestation [5–7].

Because PE can be fatal, early detection is very important, so that appropriate monitoring and clinical management can be employed. Some trials for examining PE at early and mid-gestation showed that prophylactic intervention is non-effective. However, several studies showed that prediction of PE at an early gestational age may be useful for implementing early prophylactic strategies [8].

There is no single test for predicting PE [9]. Because of the heterogenous nature of PE, there have been efforts to combine two or more biomarkers associated with different pathophysiologies to predict the development of PE [10], such as maternal serum pregnancy-associated plasma protein-A (PAPP-A), placental growth factor (PIGF), and PP-13 [11–13]. Here, biomarkers known to predict PE are reviewed.

Angiogenic Factors

1. Pro-angiogenic markers

PIGF has complicated associations with pro-angiogenic factors vascular endothelial growth factor (VEGF) and PIGF, as well as their cognate receptors VEGF receptor-1 (also known as fms-like tyrosine kinase-1 [Flt-1]) and VEGF receptor-2, which is required for angiogenesis [14].

PIGF is a member of the VEGF family and is related to angiogenesis and trophoblastic invasion of the maternal spiral arteries. Median PIGF levels in the serum show a curvilinear relationship with gestational age and increase in the first and second trimesters. This level typically reaches a maximum value at approximately 30 weeks' gestation age and then decreases. In pregnancies with fetal aneuploidies with disabled placental development, PIGF levels in the maternal serum at early pregnancy (11–13 weeks' gestation) decreases, which can result in PE and small for gestational age (SGA) neonates. PIGF levels also decrease in the second and third trimesters of pregnancy when PE is advanced or in women having SGA neonates. Thus, a relationship may exist between PE and SGA neonates [15].

2. Anti-angiogenic markers

1) Serum soluble fms-like tyrosine kinase-1

Soluble Flt-1 (sFlt-1) is a truncated splice variant of membrane-bound Flt-1. sFlt-1 circulates in the serum without limitations and binds to and neutralizes VEGF and PIGF. Previous studies revealed a relationship between increased sFlt-1 levels and

PE [16]. As early as 5 weeks before the onset of PE, sFlt-1 levels increase and remain elevated compared to unaffected pregnant women [17].

Since Maynard et al. [18] reported the contribution of excess sFlt-1, which is produced in the placenta, to the pathogenesis of PE, numerous studies have examined the usefulness of anti-angiogenic markers for diagnosing, predicting, and managing PE and placenta-related disorders.

For the pathogenesis of early-onset PE, early placentation with incomplete trophoblast invasion and spiral arteries with limited remodeling are important, and may lead to lower perfusion between the uterus and placenta. In terms of the pathogenesis of PE, the combination of second- and third-trimester sFlt-1/PIGF ratios as predictive markers of PE was examined in a low-risk group. The results showed an 87.5% detection rate with a fixed false-positive rate of 10% for early prediction of PE [19].

Pregnancy-associated Plasma Protein-A

PAPP-A is a large highly glycosylated protein and insulin-like growth factor that binds the protein protease. It is produced by developing trophoblast cells, known as syncytiotrophoblasts [20].

The insulin-like growth factor system is known to play a critical role in placental growth and development; a relationship between higher serum PAPP-A levels and an increased incidence of PE has been observed. Although decreased PAPP-A levels in the maternal serum have been reported in PE populations, these studies only examined early second trimester pregnancy [21–23]. In a multicenter study of 8,839 women, a significant relationship between PAPP-A levels below the 5th percentile and intrauterine growth retardation, preterm delivery, PE, and stillbirth were observed [24].

In addition, the significance of the levels of PAPP-A in the first trimester and sFlt-1/PIGF ratio (detection rate of 87.5% with a fixed false-positive ratio of 5%) to predict the risk of late-onset PE at the second trimester was reported [19]. Low levels of PIGF and PAPP-A in the maternal serum may reflect impaired placentation, which results in the development of PE [25].

Inhibin-A and Activin-A

Inhibin-A and activin-A are glycoproteins and members of the transforming growth factor- β family and are released by the fetoplacental unit. It is known that inhibin-A plays an essential endocrine role in the negative feedback of gonadotropins, while

activin-A is involved in various biological activities [26].

Inhibin-A and activin-A are also involved in the production of trophoblasts, and their high concentrations may reflect a placental compensatory mechanism for promoting trophoblastic invasion in situations where this procedure is damaged, and increases in women who will have PE [24]. Among the multiple markers for Down syndrome screening, mid-trimester inhibin-A levels were reported to be the best predictor of PE [27,28]. An increased serum inhibin-A level was significantly related to subsequent PE; however, inhibin-A levels showed poor sensitivity for predicting PE [26,29].

Placental Protein 13

Placental protein 13 (PP-13) is a member of the galectin super-family (defined as galectin 13); this is a family of carbohydrate-binding proteins known as β -galactoside-specific lectins in the syncytiotrophoblast [30,31].

In normal pregnancies, PP13 levels in the serum gradually increase by 2-3 folds before birth [32]. Serum PP13 levels were significantly lower in patients who developed early onset PE compared to those in normal pregnancies, which can be observed as early as 5 to 7 weeks of gestation. Therefore, maternal serum levels of PP13 in the first trimester may be a reasonable marker for PE risk assessment [33,34].

Cystatin C

Cystatin C is an established marker of renal function; when the glomerular filtration rate decreases, cystatin C levels increase [35]. In PE, cystatin C is present in the placenta and is increased at the mRNA and protein levels, and is thus elevated in the maternal plasma [36]. Median cystatin C concentrations were found to be significantly higher in a PE group (median, 0.65 mg/L) than in a control group (median, 0.57 mg/L; $P=0.0001$) in the first trimester of pregnancy [37].

Therefore, renal function assessment plays a critical role in monitoring and predicting the severity in PE. Cystatin-C, a novel marker for the detection of renal impairment, appears to be an early-stage PE marker.

Pentraxin

Pentraxin 3 (tumor necrosis factor-stimulated gene-14), which is composed of 381 amino acids, is a member of a family that includes C-reactive protein and serum amyloid P com-

ponent [38]. In PE, a maternal inflammatory response leads to higher levels of pentraxin 3 [39].

P-Selectin

P-Selectin is a member of the selectin group of cell surface adhesion molecules and is produced by activated platelets and endothelial cells. P-selectin supports the recruitment and activation of circulating leucocytes, as well as coagulation by generating leukocyte-derived "bloodborne" tissue factor, making it an important factor in inflammatory reactions [40,41].

PE is known to have extensive platelet activation activities [42-44]. P-Selectin rapidly shed from the cellular membrane of activated platelets [45] and P-selectin-exposing micro-particles with procoagulant activity were detected in the peripheral blood of women with PE [46,47]. However, there was no significant difference in P-selectin, catalase, and superoxide dismutase between PE (case group) and pregnant women with normal blood pressure (control group) in a recent study [48]. To date, various aspects of P-selectin activity were reported to be affected by PE. Thus, it is important to determine the complex interactions of P-selectin in context-specific situations of PE.

Genetic Markers for Pre-eclampsia

Genes in various biological pathways related to the immune system, control of vascular resistance, blood coagulation, cell signaling pathways, and metabolic processes, are thought to cause PE and its complications [49].

A meta-analysis in the Human Genome Epidemiology Review revealed a relatively high risk association of severe PE with the coagulation factor V gene (proaccelerin, labile factor) (F5) polymorphism rs6025 (odds ratio [OR]=1.90, 95% confidence interval [CI]: 1.42-2.54; 23 studies, $I^2=29\%$), coagulation factor II (thrombin) gene (F2) mutation G20210A (rs1799963) (OR=2.01, 95% CI: 1.14-3.55; 9 studies, $I^2=0\%$), leptin receptor gene polymorphism rs1137100 (OR=1.75, 95% CI: 1.15-2.65; 2 studies, $I^2=0\%$), and thrombophilic gene group (OR=1.87, 95% CI: 1.43-2.45; $I^2=27\%$) [49].

In another study in 2012, seven genetic variants related to PE were found: angiotensin-converting enzyme, cytotoxic T-lymphocyte-associated protein 4, factor 2, factor V (two variants), lipoprotein lipase, and serine peptidase inhibitor 1 genes. This meta-analysis suggested a relationship between PE and systems such as the renin-angiotensin system, coagulation and fibrinolysis system, and system of lipid metabolism and inflammation

[50]. However, genetic factors of PE remain unclear, and further studies are needed to determine the influence of genetic factors on PE.

Cell-free DNA

There is now a wealth of data showing that cell-free DNA (cffDNA) has immunostimulatory properties via complex molecular mechanisms [51]. Human fetal DNA modulates immunity through nuclear factor (NF)- κ B activation *in vitro* has been demonstrated by Scharfe-Nugent et al. [52], who found that NF- κ B activation resulted in the production of proinflammatory interleukin-6 in human B-cells and peripheral blood mononuclear cells. This occurs in both pregnant and non-pregnant groups. Fetal DNA is produced in the placenta. The relationship between fetal DNA and PE was recently investigated and higher fetal DNA was observed in the group with a greater risk of developing PE. Additionally, the highest level of fetal DNA was found in women with HELLP syndrome [53,54].

Lo et al. [55] reported the possibility of detecting cffDNA in the maternal plasma based on PE index. They observed that plasma cffDNA in the third trimester was increased by approximately 5-fold in 20 PE women compared to in 20 pregnant women (age-matched) controls. Many studies of using cffDNA as a predictor of PE are currently underway, and its combination with other markers has been reported as a potential predictor of PE (e.g., P-selectin, PAPP-A, PP-13, sFlt-1, and PIGF) [56]. Among 13 studies of the quantity of cffDNA suitable for predicting PE, 11 studies showed higher cffDNA in women who developed PE and 4 studies showed that cffDNA was increased in the maternal plasma before the onset of PE [57].

MicroRNAs (miRNAs) are noncoding RNA transcripts account for approximately 2% of human genes. MiRNAs are critical post-transcriptional regulators of gene expression in both healthy people and people with diseases [58]. In healthy term placenta, miRNA levels were evaluated [59–61]; in cases of placental insufficiency, miRNA levels were altered, indicating that miRNAs are useful predictors of PE [59–64]. In a study by Murphy et al. [65] in 2015, circulating miRNA profiles were identified at the time of delivery and at 1 year postpartum in PE and control groups by quantitative reverse transcription-PCR, and seven maternal plasma miRNAs (miR-98, miR-222, miR-210, miR-155, miR-296, miR-181a, and miR-29b) were increased in women with severe PE. Recent studies of placenta-specific miRNAs in the maternal circulation also indicated their potential as predictive markers of placental insufficiency [66–69].

Conclusion

PE is well known as an important disease related to maternal morbidity and mortality. Many studies have attempted to identify the causes of PE. While many factors involved in the pathophysiology of PE have been suggested, the precise causes of PE remain unknown.

Many different factors are related to the pathophysiology of PE [70]. Some of these factors can help clinicians predict PE and enable management to begin in the first trimester. However, further studies of the causes of PE are needed, which help to predict PE earlier and more accurately improving treatment.

Thus, additional studies are needed to elucidate the molecular biomarkers and genetics, such as DNA and miRNA for predicting PE. Additionally, large-scale, multicenter, multi-ethnic, prospective trials considering different possibilities to develop PE prediction are required to identify better combinations of markers for screening PE.

References

1. Duley L. The global impact of pre-eclampsia and eclampsia. *Semin Perinatol* 2009;33:130–7.
2. Spencer-Jones J. Make every mother and child count. *S Afr Med J* 2005;95:382, 384.
3. Lyons G. Saving mothers' lives: confidential enquiry into maternal and child health 2003–5. *Int J Obstet Anesth* 2008;17:103–5.
4. Warrington JP, George EM, Palei AC, Spradley FT, Granger JP. Recent advances in the understanding of the pathophysiology of preeclampsia. *Hypertension* 2013;62:666–73.
5. Irgens HU, Reisaeter L, Irgens LM, Lie RT. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. *BMJ* 2001;323:1213–7.
6. Yu CK, Khouri O, Onwudiwe N, Spiliopoulos Y, Nicolaidis KH; Fetal Medicine Foundation Second-Trimester Screening Group. Prediction of pre-eclampsia by uterine artery Doppler imaging: relationship to gestational age at delivery and small-for-gestational age. *Ultrasound Obstet Gynecol* 2008;31:310–3.
7. Witlin AG, Saade GR, Mattar F, Sibai BM. Predictors of neonatal outcome in women with severe pre-eclampsia or eclampsia between 24 and 33 weeks' gestation. *Am J Obstet Gynecol* 2000;182:607–11.
8. Thilaganathan B, Wormald B, Zanardini C, Sheldon J, Ralph E, Pappageorghiou AT. Early-pregnancy multiple serum markers and second-trimester uterine artery Doppler in predicting preeclampsia. *Obstet Gynecol* 2010;115:1233–8.
9. Zhong Y, Tuuli M, Odibo AO. First-trimester assessment of placenta

- function and the prediction of preeclampsia and intrauterine growth restriction. *Prenat Diagn* 2010;30:293-308.
10. Sczaccchio E, Figueras F. Contemporary prediction of preeclampsia. *Curr Opin Obstet Gynecol* 2011;23:65-71.
 11. Cuckle HS. Screening for pre-eclampsia--lessons from aneuploidy screening. *Placenta* 2011;32 Suppl:S42-8.
 12. Nicolaides KH, Bindra R, Turan OM, Chefetz I, Sammar M, Meiri H, et al. A novel approach to first-trimester screening for early pre-eclampsia combining serum PP-13 and Doppler ultrasound. *Ultrasound Obstet Gynecol* 2006;27:13-7.
 13. Anderson UD, Gram M, Jälmy M, Kerström B, Hansson SR. [167-POS] : The human endogenous protection system against cell-free hemoglobin is overwhelmed during preeclampsia - New biomarkers and potential targets for therapy. *Pregnancy Hypertens* 2015;5:86.
 14. Tjwa M, Luttun A, Autiero M, Carmeliet P. VEGF and PlGF: two pleiotropic growth factors with distinct roles in development and homeostasis. *Cell Tissue Res* 2003;314:5-14.
 15. Tsiakkas A, Duvdevani N, Wright A, Wright D, Nicolaides KH. Serum placental growth factor in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol* 2015;45:591-8.
 16. Kar M. Role of biomarkers in early detection of preeclampsia. *J Clin Diagn Res* 2014;8:BE01-4.
 17. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004;350:672-83.
 18. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003;111:649-58.
 19. Park HJ, Kim SH, Jung YW, Shim SS, Kim JY, Cho YK, et al. Screening models using multiple markers for early detection of late-onset preeclampsia in low-risk pregnancy. *BMC Pregnancy Childbirth* 2014;14:35.
 20. Lawrence JB, Oxvig C, Overgaard MT, Sottrup-Jensen L, Gleich GJ, Hays LG, et al. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proc Natl Acad Sci U S A* 1999;96:3149-53.
 21. Deveci K, Sogut E, Evliyaoglu O, Duras N. Pregnancy-associated plasma protein-A and C-reactive protein levels in pre-eclamptic and normotensive pregnant women at third trimester. *J Obstet Gynaecol Res* 2009;35:94-8.
 22. Bersinger NA, Ødegård RA. Second- and third-trimester serum levels of placental proteins in preeclampsia and small-for-gestational age pregnancies. *Acta Obstet Gynecol Scand* 2004;83:37-45.
 23. Bersinger NA, Smáráson AK, Muttukrishna S, Groome NP, Redman CW. Women with preeclampsia have increased serum levels of pregnancy-associated plasma protein A (PAPP-A), inhibin A, activin A and soluble E-selectin. *Hypertens Pregnancy* 2003;22:45-55.
 24. Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. Early pregnancy levels of pregnancy-associated plasma protein A and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. *J Clin Endocrinol Metab* 2002;87:1762-7.
 25. Poon LC, Akolekar R, Lachmann R, Beta J, Nicolaides KH. Hypertensive disorders in pregnancy: screening by biophysical and biochemical markers at 11-13 weeks. *Ultrasound Obstet Gynecol* 2010;35:662-70.
 26. Muttukrishna S, North RA, Morris J, Schellenberg JC, Taylor RS, Asselin J, et al. Serum inhibin A and activin A are elevated prior to the onset of pre-eclampsia. *Hum Reprod* 2000;15:1640-5.
 27. Kang JH, Farina A, Park JH, Kim SH, Kim JY, Rizzo N, et al. Down syndrome biochemical markers and screening for preeclampsia at first and second trimester: correlation with the week of onset and the severity. *Prenat Diagn* 2008;28:704-9.
 28. Ree PH, Hahn WB, Chang SW, Jung SH, Kang JH, Cha DH, et al. Early detection of preeclampsia using inhibin A and other second-trimester serum markers. *Fetal Diagn Ther* 2011;29:280-6.
 29. Sibai BM, Koch MA, Freire S, Pinto e Silva JL, Rudge MV, Martins-Costa S, et al. Serum inhibin A and angiogenic factor levels in pregnancies with previous preeclampsia and/or chronic hypertension: are they useful markers for prediction of subsequent preeclampsia? *Am J Obstet Gynecol* 2008;199:268.e1-9.
 30. Than NG, Romero R, Goodman M, Weckle A, Xing J, Dong Z, et al. A primate subfamily of galectins expressed at the maternal-fetal interface that promote immune cell death. *Proc Natl Acad Sci U S A* 2009;106:9731-6.
 31. Than NG, Romero R, Kim CJ, McGowen MR, Papp Z, Wildman DE. Galectins: guardians of eutherian pregnancy at the maternal-fetal interface. *Trends Endocrinol Metab* 2012;23:23-31.
 32. Huppertz B. Placental origins of preeclampsia: challenging the current hypothesis. *Hypertension* 2008;51:970-5.
 33. Huppertz B, Sammar M, Chefetz I, Neumaier-Wagner P, Bartz C, Meiri H. Longitudinal determination of serum placental protein 13 during development of preeclampsia. *Fetal Diagn Ther* 2008;24:230-6.
 34. Gonen R, Shahar R, Grimpel YI, Chefetz I, Sammar M, Meiri H, et al. Placental protein 13 as an early marker for pre-eclampsia: a prospective longitudinal study. *BJOG* 2008;115:1465-72.
 35. Grubb AO. Cystatin C--properties and use as diagnostic marker. *Adv Clin Chem* 2000;35:63-99.
 36. Kristensen K, Larsson I, Hansson SR. Increased cystatin C expression in the pre-eclamptic placenta. *Mol Hum Reprod* 2007;13:189-95.
 37. Thilaganathan B, Ralph E, Papageorgiou AT, Melchiorre K, Sheldon

- J. Raised maternal serum cystatin C: an early pregnancy marker for preeclampsia. *Reprod Sci* 2009;16:788-93.
38. Souza DG, Soares AC, Pinho V, Torloni H, Reis LF, Teixeira MM, et al. Increased mortality and inflammation in tumor necrosis factor-stimulated gene-14 transgenic mice after ischemia and reperfusion injury. *Am J Pathol* 2002;160:1755-65.
39. Cetin I, Cozzi V, Pasqualini F, Nebuloni M, Garlanda C, Vago L, et al. Elevated maternal levels of the long pentraxin 3 (PTX3) in pre-eclampsia and intrauterine growth restriction. *Am J Obstet Gynecol* 2006;194:1347-53.
40. André P. P-selectin in haemostasis. *Br J Haematol* 2004;126:298-306.
41. Polgar J, Matuskova J, Wagner DD. The P-selectin, tissue factor, coagulation triad. *J Thromb Haemost* 2005;3:1590-6.
42. Holthe MR, Staff AC, Berge LN, Lyberg T. Different levels of platelet activation in preeclamptic, normotensive pregnant, and nonpregnant women. *Am J Obstet Gynecol* 2004;190:1128-34.
43. Konijnenberg A, Stokkers EW, van der Post JA, Schaap MC, Boer K, Bleker OP, et al. Extensive platelet activation in preeclampsia compared with normal pregnancy: enhanced expression of cell adhesion molecules. *Am J Obstet Gynecol* 1997;176:461-9.
44. Yoneyama Y, Suzuki S, Sawa R, Kiyokawa Y, Power GG, Araki T. Plasma adenosine levels and P-selectin expression on platelets in preeclampsia. *Obstet Gynecol* 2001;97:366-70.
45. Dunlop LC, Skinner MP, Bendall LJ, Favalaro EJ, Castaldi PA, Gorman JJ, et al. Characterization of GMP-140 (P-selectin) as a circulating plasma protein. *J Exp Med* 1992;175:1147-50.
46. Bretelle F, Sabatier F, Desprez D, Camoin L, Grunebaum L, Combes V, et al. Circulating microparticles: a marker of procoagulant state in normal pregnancy and pregnancy complicated by preeclampsia or intrauterine growth restriction. *Thromb Haemost* 2003;89:486-92.
47. Lok CA, Nieuwland R, Sturk A, Hau CM, Boer K, Vanbavel E, et al. Microparticle-associated P-selectin reflects platelet activation in preeclampsia. *Platelets* 2007;18:68-72.
48. Nasrollahi S, Hoseini Panah SM, Tavilani H, Tavasoli S, Naderan M, Shoar S. Antioxidant status and serum levels of selectins in preeclampsia. *J Obstet Gynaecol* 2015;35:16-8.
49. Fong FM, Sahemey MK, Hamed G, Eytayo R, Yates D, Kuan V, et al. Maternal genotype and severe preeclampsia: a HuGE review. *Am J Epidemiol* 2014;180:335-45.
50. Buurma AJ, Turner RJ, Driessen JH, Mooyaart AL, Schoones JW, Bruijn JA, et al. Genetic variants in pre-eclampsia: a meta-analysis. *Hum Reprod Update* 2013;19:289-303.
51. Isaacs A, Cox RA, Rotem Z. Foreign nucleic acids as the stimulus to make interferon. *Lancet* 1963;2:113-6.
52. Scharfe-Nugent A, Corr SC, Carpenter SB, Keogh L, Doyle B, Martin C, et al. TLR9 provokes inflammation in response to fetal DNA: mechanism for fetal loss in preterm birth and preeclampsia. *J Immunol* 2012;188:5706-12.
53. Miranda ML, Macher HC, Muñoz-Hernández R, Vallejo-Vaz A, Moreno-Luna R, Villar J, et al. Role of circulating cell-free DNA levels in patients with severe preeclampsia and HELLP syndrome. *Am J Hypertens* 2013;26:1377-80.
54. Tjoa ML, Cindrova-Davies T, Spasic-Boskovic O, Bianchi DW, Burton GJ. Trophoblastic oxidative stress and the release of cell-free fetal-placental DNA. *Am J Pathol* 2006;169:400-4.
55. Lo YM, Leung TN, Tein MS, Sargent IL, Zhang J, Lau TK, et al. Quantitative abnormalities of fetal DNA in maternal serum in preeclampsia. *Clin Chem* 1999;45:184-8.
56. Grill S, Rusterholz C, Zanetti-Dällenbach R, Tercanli S, Holzgreve W, Hahn S, et al. Potential markers of preeclampsia—a review. *Reprod Biol Endocrinol* 2009;7:70.
57. Martin A, Krishna I, Badell M, Samuel A. Can the quantity of cell-free fetal DNA predict preeclampsia: a systematic review. *Prenat Diagn* 2014;34:685-91.
58. Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. *Development* 2005;132:4653-62.
59. Enquobahrie DA, Abetew DF, Sorensen TK, Willoughby D, Chidambaram K, Williams MA. Placental microRNA expression in pregnancies complicated by preeclampsia. *Am J Obstet Gynecol* 2011;204:178.e12-21.
60. Mayor-Lynn K, Toloubeydokhti T, Cruz AC, Chegini N. Expression profile of microRNAs and mRNAs in human placentas from pregnancies complicated by preeclampsia and preterm labor. *Reprod Sci* 2011;18:46-56.
61. Wang W, Feng L, Zhang H, Hachy S, Satohisa S, Laurent LC, et al. Preeclampsia up-regulates angiogenesis-associated microRNA (i.e., miR-17, -20a, and -20b) that target ephrin-B2 and EPHB4 in human placenta. *J Clin Endocrinol Metab* 2012;97:E1051-9.
62. Choi SY, Yun J, Lee OJ, Han HS, Yeo MK, Lee MA, et al. MicroRNA expression profiles in placenta with severe preeclampsia using a PNA-based microarray. *Placenta* 2013;34:799-804.
63. Pineles BL, Romero R, Montenegro D, Tarca AL, Han YM, Kim YM, et al. Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. *Am J Obstet Gynecol* 2007;196:261.e1-6.
64. Zhu XM, Han T, Sargent IL, Yin GW, Yao YQ. Differential expression profile of microRNAs in human placentas from preeclamptic pregnancies vs normal pregnancies. *Am J Obstet Gynecol* 2009;200:661.e1-7.
65. Murphy MS, Casselman RC, Tayade C, Smith GN. Differential expression of plasma microRNA in preeclamptic patients at delivery and 1 year postpartum. *Am J Obstet Gynecol* 2015;213:367.e1-9.

66. Hromadnikova I. Extracellular nucleic acids in maternal circulation as potential biomarkers for placental insufficiency. *DNA Cell Biol* 2012;31:1221-32.
67. Hromadnikova I, Kotlabova K, Doucha J, Dlouha K, Krofta L. Absolute and relative quantification of placenta-specific micrnas in maternal circulation with placental insufficiency-related complications. *J Mol Diagn* 2012;14:160-7.
68. Pan M, Ge Q, Li H, Yang Q, Lu J, Zhang D, et al. Sequencing the miRNAs in maternal plasma from women before and after parturition. *J Nanosci Nanotechnol* 2012;12:4035-43.
69. Chim SS, Shing TK, Hung EC, Leung TY, Lau TK, Chiu RW, et al. Detection and characterization of placental microRNAs in maternal plasma. *Clin Chem* 2008;54:482-90.
70. Roberts JM, Bell MJ. If we know so much about preeclampsia, why haven't we cured the disease? *J Reprod Immunol* 2013;99:1-9.