**Name of journal: World Journal of Obstetrics and Gynecology**

**ESPS Manuscript NO: 9832**

**Columns: Review**

Preeclampsia: Definitions and screening tools and diagnostic criteria in the supersonic era

Montagnoli C *et al*. Possible new markers for screening of preeclampsia

**Carlotta Montagnoli, Larciprete Giovanni,**

**Carlotta Montagnoli, Larciprete Giovanni,** Department of Obstetrics and Gynaecology, Fatebenefratelli Isola Tiberina Hospital, 39 00186 Rome, Italy

**Author contributions:** Montagnoli C and Giovanni L solely contributed to this paper.

**Correspondence to: Larciprete Giovanni, MD, PhD,** Department of Obstetrics and Gynaecology Fatebenefratelli Isola Tiberina Hospital, Isola Tiberina, 39 00186 Rome, Italy. giovanni.larciprete@fbf-isola.it

**Telephone:** +39-06-6837416 **Fax:** +39-06-68214220

**Received:** February 28, 2014 **Revised:** April 15, 2014

**Accepted:**May 16, 2014

**Published online:**

**Abstract**

Preeclampsia still represents a major risk factor for maternal-fetal health. Therefore, early identification of pregnant women at risk for preeclampsia is a very priority in obstetric, to decrease the mortality and morbidity associated with this disease. On the basis of well-known and new pathophysiological mechanisms of preeclampsia, in literature it been investigated different biochemical and ultrasonographic parameters, without, however, find an ideal marker for early screening. In this brief review we present the best studied ultrasonographic markers, and the most new genetic factors and promising emerging biomarkers of preeclampsia, to date. We hope that in the future the combination of these several tests will allow us to predict women at risk of preeclampsia.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words**: Preeclampsia; Diagnosis of preeclampsia; Screening of preeclampsia; Ultrasonographic markers of preeclampsia

**Core tip:** Preeclampsia is a very important pregnancy disease, but in its management has been demonstrated a substandard care. The core contents of this paper is the review of the literature to evaluate possible markers for early diagnosis of preeclampsia.

Montagnoli C, Giovanni L.Preeclampsia: Definitions and screening tools and diagnostic criteria in the supersonic era. *World J Obstet Gynecol* 2014; In press

**Available from: URL:**

**DOI:**

**INTRODUCTION**

The preeclampsia in still an important cause of maternal and fetal death. The Eighth report of the Confidential Enquiries into Maternal Deaths in the United Kingdom referred, in the triennium 2006-2008, 261 women died for complication related directly or indirectly to pregnancy. Among these, 22 died women were related to preeclampsia, and 20 of 22 cases demonstrated substandard care[1].

Moreover preeclampsia represents an important risk for the health of the baby. In the Perinatal Mortality Report of the United Kingdom[2] it is reported that 5% of stillbirths without congenital abnormality occurred in women with preeclampsia and that the half of women with severe preeclampsia give birth preterm.

Then a question arises: which is the real problem? To find out a univocal definition of this complex disease? Or to find out markers for the screening of preeclampsia? Or the problem is in the inadequate treatment?

In this review we point out our attention on the possibility of screening for preeclampsia basing on the data available in the literature.

However the present report can't miss the definition of preeclampsia.

For a long time there was confusion about the definition of hypertensive disorders in pregnancy.

In year 2001 the International Society for the Study of Hypertension in Pregnancy (ISSHP)[3] provides a consensus on classification, adopting the statement of the Australasian Society for the Study of Hypertension in Pregnancy (ASSHP)[4] and the report of the National High Blood Pressure Education Program (NHBPE)[5].

The definition and the classification is the fallowing: Hypertension in pregnancy, systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg.

Four categories have been identified: (1) Preeclampsia: De novo hypertension after 20 weeks’ gestation associated to proteinuria. Proteinuria is defined as appearance of urinary protein greater than 300 mg/day or a spot urine protein/creatinine ratio ≥ 30 mg/mmol; (2) Gestational hypertension: De novo hypertension alone, after 20 gestational weeks; (3) Chronic hypertension: Hypertension diagnosed before 20 weeks’ gestation or preconceptional hypertension; and (4) Preeclampsia superimposed on chronic hypertension: In a woman with chronic hypertension, development of proteinuria and/or symptoms associated with preeclampsia after 20 gestational weeks.

Both the ASSHP and NHBPEP in the definition of hypertension don't provide an increase in blood pressure of 15 mmHg and 30 mmHg, respectively, for diastolic and systolic levels but they consider values below 140/90 mmHg as absolute values.

Both the ASSHP and NHBPEP agree on the definition and classification of hypertensive disorders during pregnancy, but there is an important difference: the NHBPEP considers as diagnostic criteria only the hypertension associated to the proteinuria, whereas the ASSHP reports a clinical classification based on pathophysiology of the disorder[4]. In fact the definition of preeclampsia comprises besides hypertension and proteinuria, also renal insufficiency, liver disease, neurological problems, haematological disturbances and fetal growth restriction.

In 2009 The American Society of Hypertension published a position paper that summarized the definitions and the clinical features regarding the different forms of hypertension during pregnancy[6] and included the guideline of the American College of Obstetricians and Gynecologist.

Like other opinions, the ASH position paper considers the hypertension as a SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg, avoiding the dated concept of the increase in DBP of 15 mmHg or more and increase in SBP of 30 mmHg or more. In the definition of preeclampsia, the proteinuria is defined by appearance of urinary protein greater than 300 mg/day or a spot urine protein/creatinine ratio ≥ 30 mg/mmol or a qualitative dipstick +1. Because the dipstick has many false-positive and false-nagative, and because in pregnancy a urine collection may be difficult, the ratio proteine/creatinine is recommended in the ASH position paper.

Also the terminology used is that recommended by NHBPEP that provides: Preeclampsia/ eclampsia, Gestational hypertension, Chronic hypertension and preeclampsia superimposed on chronic hypertension.

However, the ASH position paper introduces also new entities: (1) Late post-partum hypertension: usually, in preeclamptic or in women with hypertensive disorders in pregnancy, the blood pressure returns normal in the immediate post-partum. However there is a little-known entity in which the hypertension appears after delivery in women with normotensive gestation and regresses within first post-partum year; (2) Late post-partum eclampsia: the eclamptic convulsions occur from 48 h to several weeks after delivery; and (3) Early gestational hypertension: this is a very rare entity in which patients have excessive sensitivity to progesterone due to activating mineralcorticoid reception mutations. These women develop early hypertension concomitantly with the progesterone rise in the first trimester.

Preeclampsia in the ASH position paper is usually defined by hypertension associated to proteinuria, but on the basis of symptoms, hypertension level and clinical data, the American Society of Hypertension suggests the distinction between "Less Severe" and "More Severe" preeclampsia (defined by the American College of Ostetrics and Gynecology-ACOG, as Mild and Severe preeclampsia). The "more severe preeclampsia" is defined in presence of severe hypertension (≥ 110 mmHg diastolic and ≥ 160 mmHg systolic), nephrotic range proteinuria, oliguria, neurologic symptoms, thrombocytopenia (< 100.000/µL), haemolysis or abnormal liver function.

Despite this distinction, the American Society of Hypertension recommended that even only a suspicion of preeclampsia is a sufficient reason for hospitalization, because all preeclampsia is potentially explosive.

In 2010 the National Institute for Health and Clinical Excellence (NICE)[7] published a guideline including a classification. The definition and classifications are as follows: (1) Chronic hypertension: hypertension that is present at the booking visit or before 20 weeks’ gestation. It can be primary or secondary in aetiology; (2) Gestational hypertension: it is a new hypertension presenting after 20 weeks’ gestation without significant proteinuria; (3) Preeclampsia: it is a new hypertension presenting after 20 weeks’ gestation with significant proteinuria. Significant proteinuria is defined as +1 or more in an automated reagent strip or urinary pretein/creatinine ratio greater that 30mg/mmol or greater that 300 mg protein in 24-h urine collections; (4) Eclampsia: it is a convulsive condition associated with preeclampsia; (5) HELLP Syndrome: haemolysis, elevated liver enzymes and low platelet count; (6) Severe preeclampsia: preeclampsia with severe hypertension and/or with symptoms, and/or biochemical and/or haematological impairment. In addition the Guideline Development Group (GDG) has defined three different levels of hypertension: mild, moderate and severe; (7) Mild hypertension: diastolic BP 90-99 mmHg, systolic BP 140-149 mmHg; (8) Moderate hypertension: diastolic BP 100-109 mmHg, systolic BP 150-159 mmHg; and (9) Severe hypertension: diastolic BP 110 mmHg or greater, systolic BP 160 mmHg or greater.

According with the ASH position paper, the NICE guideline recommended hospitalization of preeclamptic women with all degree of hypertension.

The 2010 guidelines of the Royal College of Obstetrics and Gynecologist (RCOG)[8] (regarding the Management of Severe Preeclampsia, substantially agree with the other definitions of preeclampsia, but differences still remain with the NICE guidelines about the definition of severe preeclampsia. The RCOG considers severe preeclampsia as the presence of a DBP ≥ 110 mmHg on two occasions or a SBP ≥ 170 mmHg on two measurements, with signficant proteinuria (at least 1 g/L).

According with the ASH position paper, the RCOG guidelines reported the Evidence level Ib and level IIb regarding the measurement of blood pressure, which are referred below.

The women should be rested and sitting at a 45-degree angle. The cuff should be of appropriate size and be placed at the level of heart. The diastolic pressure is designed at 5th Korotkoff phase, therefore the older concept that the gravid women show large differences between the 4th and the 5th Korotkoff phase has been abandoned and the 5th Korotkoff has been established as the sound of true diastolic pressure.

**SCREENING FOR PREECLAMPSIA**

***Ultrasounds***

**Uterine artery Doppler**: The increase of impedance to flow in the uterine artery is an evidence of impaired throphoblastic invasion of the maternal spiral arteries and this is a well-known mechanism of the preeclampsia’s pathophysiology. In fact several studies was shown a reduction in the maternal uterine resistance index with advancing gestational age in normal pregnancy[9], while the presence of an increased resistance in maternal flow or the presence of a notch, as evidence of abnormal uterine flow, has been associated with the development of preeclampsia. Already for many years, the Doppler ultrasound evaluation of uterine arteries was used to predict an unfavorable pregnancy outcome. However in the literature discrepant results are described among the studies (Table 1), which could be due to the different gestational age at witch the women were evaluated, to different populations included, to the single or two steps examination, to the different cut-off of abnormal resistance index and finally to the differences in US Doppler technique.

In an unselected population Bower *et al*[15], considering women at 18-22 weeks’gestation with abnormal resistance index (above the 95th centile and/or with the presence of a notch within the uterine artery Doppler waveform), reported a sensitivity of 75% and specificity of 86% for preeclampsia, with a better prediction for severe conditions. Valensise *et al*[15] in primigravidas at 22 weeks’ gestation with increased impedance (resistance index more than 0.58) found a sensitivity of 74% and specificity of 97,5% for the development of gestational hypertension.

Other authors report less favorable results. Chan *et al*[16] in women at 20 weeks’gestation found that sensitivity of the test for preeclampsia was 27**%** and specificity was 97%. Similar results from North *et al*[17] that found at 19-24 weeks’ gestation a sensitivity of 27% and specificity of 90%.

With the aim of reducing the number of false-positive Steel *et al*[18] proposed a two step trial for uterine Doppler US: the first evaluation at 18 weeks’s gestation and in presence of increased impedance (resistance index greater that 0.58), a secondo Doppler evaluation at 24 weeks’gestation. The author reported a sensitivity for preeclampsia of 63%. Also Bower *et al*[19] reported an increase of positive predictive value for preeclampsia from 12% to 28%, reanalysing at 24 wk women found with abnormal Doppler US at 20 weeks’ gestation.

Several studies regarding the application of Doppler uterine evaluation as a screening tool are conducted in selected population at risk for preeclampsia. Arduini *et al*[13] evaluated women with previous gestational hypertensive disorders or essential chronic hypertension, at 18-20 wk and reported a sensitivity of 64% and a specificity of 94%, but the true value of those data are still questionable. In fact several bias and criticisms have been found from data belonging to this research group. Jacobson *et al*[12] , examining women with chronic hypertension or history of preeclampsia, found a sensitivity of 44% and specificity of 73% for preeclampsia. Also Caruso *et al*[20] in order to assess the predictivity power of Doppler uterine US, examined women with chronic hypertension and he referred a sensitivity of 78% and specificity of 45%.

Valensise *et al*[11] in his paramount study observed that the value of Doppler uterine evaluation as screening test strictly depends on the studied population: concerning the high risk population, the positive predictive value for hypertension is acceptable for the screening, while in low risk pregnant women the correlation seems to be weaker. More recently Elena Parretti from Florence[21] in normotensive women with risk factors for preeclampsia, conduced a cross-sectional (at 24 weeks’ gestation) and a longitudinal (at 16, 20 and 24 weeks’ gestation) study of uterine artery Doppler. In agreement with the other investigators, that study confirmed the value of 0.58 as normal resistance index and obtained a positive predictive value of 44%, still inadequate for a screening test. Instead, with a longitudinal approach, it seemed to improve the positive predictive value to 72.2%, by reducing a number of false-positive results.

To improve the possibility to use the Doppler velocimetry of the uterine arteries as a screening for preeclampsia, several studies proposed other parameters likely to be integrated with the Doppler evaluation.

Valensise *et al*[22] proposed the combination of Doppler and 24-h automated maternal blood pressure evaluation. This study stated that in presence of abnormal Doppler and asyntomatic increased blood pressure, patients had higher incidence of pregnancy complications with a positive predictive value of 76% for preeclampsia.

With the aim to reduce the number of false-positive patients, other authors have proposed to use the Doppler velocimetry associated with biochemical parameters.

Elevated level of second trimester β-human chorionic gonadotropin have been found in plasma of patients at risk for hypertensive disorders in pregnancy[23]. A study of Elsandabesee *et al*[24] demonstrated that in presence of diastolic notch, the association of serum screening with alfa-feto-protein and β-human chorionic gonadotropin, improves sensitivity and positive predictive value to 91% and 41% respectively.

Regarding the PP-13, initial studies showed a significantly decrease in preeclamptic women[25,26], while recently Stamatopoulou *et al*[27] did not show a relationship between PP-13 levels and preeclampsia. Akolekar *et al*[28] studied placental protein-13 (PP-13) associated with pregnancy associated pregnancy protein-A (PAPP-A) and uterine artery Doppler US in the first trimester in 208 preeclamptic patients and in 416 normal pregnancies showing a significant reduction of PP-13 level in early preeclampsia but not in late preeclampsia with a positive predictive value for early preeclampsia of 79%, while for late preeclampsia of 49%. Though in preeclampsia PAPP-A was reduced and uterine velocimetry Doppler was increased, the combination of these parameters with PP-13 does not look to improve the sensitivity of PP-13[28].

A systematic review in 2010[29] studied the role of biochemical markers associated with ultrasonographic markers to improve the possibility of prediction for early preeclampsia. The authors included within their review 37 articles in which the most frequently studied biochemical markers were hCG (human chorionic gonadotropin), inhibin A, alfa-fetoprotein, sFlt-1 (soluble fms-like tyrosine kinase 1), PAPP-A (pregnancy associated plasma protein-A), activin A, PlGF (placental growth factor) and PP-13 (placental protein-13). In some cases markers were evaluated in the second trimester as well as the ultrasound velocimetry, in other cases the markers were assessed during the first trimester, before ultrasonographic evaluation. The analysis of this papers elucidates that the addition of biochemical markers to uterine artery Doppler ultrasound scan in the second trimester or the combination of first trimester biochemical and second trimester uterine velocimetry improves the predictive performance of ultrasound alone and of markers alone. Not only but this review suggests that the addition of maternal characteristics does improve their predictive power.

Despite the promising results, the heterogeneity between studies regarding gestational age at the study time or the selected populations (high versus low risk), led us to incertitude towards the combination of ultrasonographic and biochemical markers as screening procedure for preeclampsia.

**Maternal echocardiography:** It's well known that in pregnancy there important changes occur in haemodynamic and cardiovascular system with initial vasodilatative adaptation of maternal cardiovascular tree that begins in the first trimester as a consequence of invasion of the spiral arteries by the throphoblast. Indeed the remodelling of the spiral arteries contributes from 20% to 26% to the total reduction of systemic vascular resistance in the second trimester[30]. Another important change is in the body composition with increase in blood volume. A study based on the multifrequency bioelectrical impedance, documented that the total body water, extracellular water and intracellular water increased significantly and progressively from the first to the second trimester[31].

Cardiovascular and haemodynamic modifications consist of an increased pre-load, a decreased after-load, an increased compliance of the vascular tree and a ventricular remodelling at the level of the heart. We have therefore an enlargement of the vascular bed and an increase in blood volume to fill the enlarged vascular bed. Conversely, an inadequate placentation and the failure of the haemodynamic adaptation was identified to the base of the pathologic process that leads to pregnancy complications. Already in 1988 Nisell *et al*[32] showed that in preeclamptic women, independently from the cardial output, a high peripheral resistance can be observed and in those with a low cardiac output generally a low birthweight could occur. Duvekot *et al*[33] observed that patients with fetal growth restriction (FGR) had a smaller left atrial diameter and a failure of cardiac output in early pregnancy.

On this basis, the study group of Valensise designed a different study to evaluate the predictive value of some echocardiographic parameters for maternal and fetal complications, alone or associated with uterine doppler velocimetry[30,34].

The same author[30] in his first study on this topic has evaluated the relationship between cardiac systolic and diastolic function and uteroplacental resistance in a longitudinal observation of 248 patients with a normal pregnancy. Concerning the uterine Doppler velocimetry, he reported a significant reduction in resistance index between first and second trimester. The ecocardiographic evaluation showed in normal pregnant women a significant increase in left atrial diameter, stroke volume and cardiac output throught gestation, mainly from the first to the second trimester, according with fall of uterine resistance index that contributes to decrease of the afterload.

Conversely, in a study[35] performed on 21 pregnancy complicated by gestational hypertension, the analysis of systolic and diastolic function associated with morphological left ventricular modifications showed that hypertensive women have an altered geometric pattern with concentric hypertrophy. Functionally, this finding is associated with higher blood pressure, higher total vascular resistance (TVR) and uterine resistance index versus the normotensive patients. Therefore, they recommend the use of maternal cardiac function evaluation in women presenting an abnormal uterine Doppler resistance index in the second trimester, to increase the prediction of hypertensive disorders of pregnancy. With the scope to increase the predictive values for gestational hypertension of ultrasound evaluation, Valensise *et al*[36] submitted to echocardiography 36 women with uterine Doppler abnormalities (bilateral notch and RI > 0.58) at 24 weeks’ gestation, showing in the normotensive women group a normal ventricular left isovolumetric relaxation time (IVRT), evidence of adequate diastolic function; while in patients with pathological outcomes, an elevated IVRT, meaning cardiac diastolic dysfunction and an altered ventricular geometric pattern, evidence of abnormal cardiac adaptation to pregnancy. Therefore he proposed the association of data from maternal cardiovascular adaptation with uterine artery screening to reduce the number of false-positive diagnosis of pathologic pregnancy.

In a subsequent study[37], the same research group, evaluated the predictive value for maternal and fetal complications of total vascular resistance (TVR) and left ventricular morphology in normotensive high risk primigravidas with bilateral notch of uterine artery at 24 wk. They reported that the increase of total vascular resistance above the cut-off took sensitivity at 89%, specificity at 94%, positive predictive value (PPV) at 77% and negative predictive value (NPV) at 97%. Considering the importance of the assessment of the cardiac function in pregnancy, another study[34] was conducted to evaluate the significance of myocardial function associated with abnormal uterine Doppler velocimetry, in women with hypertensive complications or in normal pregnancy. The results showed that in pregnancy with abnormal uterine arteries Doppler and complicated outcomes, the myocardial function is impaired prior to the development of complications and still remains depressed 6 mo post-partum; in women with normal uterine artery and normal pregnancy the myocardial function was unchanged compared with the post-partum; instead, in patients with bilateral notching and normal outcome of pregnancy, an enhanced myocardial function is reported and the authors hypothesize that it is a crucial mechanism to maintain normal haemodynamic parameters.

Echocardiographic parameters of cardiac performance during pregnancy could be, in normotensive women, a important predictor of pregnancy complications and of predisposition to cardiovascular disease.

***Genetic assessment***

Preeclampsia is a complex multisystem and multifactorial disorder, whose genetic component is not yet clear. However it can be hypothesized that well known aetiologic factors behind may have a genetic implication[38]. In the past, it has been suggested that Mendelian or mitochondrial gene transmission could be cause of preeclampsia, however studies conducted on monozygotic twins did not confirm this hypothesis. Also fetal genotype was investigated without demonstrating a clear role in determining an increased risk of preeclampsia[38].

Not only the genotype but also the m-RNA expression of specific genes seems to be associated with the development of preeclampsia[38]. Indeed Rajakumar *et al*[39] in a recent study, analysing leukocite gene expression, identified 368 genes differentially expressed in preeclamptic women and normotensive patients. Particularly he observed that this different expression concerns genes that play a central role in functions as cells proliferation, inflammation, apoptosis, immune function and angiogenesis, that are involved in the pathogenesis of preeclampsia.

Therefore, seem that preeclampsia is a complex disease, multifactorial and multigenic.

In a systematic review, Mutze *et al*[38] reported more that 50 candidate genes as predisposing factors for preeclampsia, but only few genes account for about 70% of research.

Evaluating a current state of the literature regarding the role of gene polymorphisms in preeclampsia we distinguish different genes on the basis of their pathophysiologyc role in this disease: endothelial dysfunction, oxidative stress and placental thrombosis.

**Genes involved in endothelial dysfunction:** Different genes were identified in endothelial remodelling and their polymorphisms have been associated to endothelial dysfunction, although with controversial results.

For example it is well known that Endothelin-1 (ET-1) is an important vasoconstrictor produced by endothelial and smooth muscle cells, as well as that endothelial -1 converting enzyme (ECE-1) is connected with ET-1 concentration. However one study examined the role of polymorphism Lys198As in the ET-1 in preeclamptic women but found no significant association[40]. Another recent study[41] evaluated the polymorphism Lys198Asn of ET-1 and Thr34IIe of ECE-1 and no statistically significant differences in polymorphic frequencies between hypertensive pregnant women and control group were collected. Moreover the gene encoding for Endothelin-1 receptor was investigated but the polymorphism considered (231G>A) was not found to be related to the risk of preeclampsia[42].

Also genes components of Renina Angiotensina Systems (RAS), as genes encoding for information regarding blood pressure, hemodynamic changes and vascular remodelling, have long been investigated to evaluate the presence of polymorphisms candidates for involvement in preeclampsia.

The polymorphism in intron 16 (insertion/deletion) of Angiotensin converting-enzyme (ACE gene) is associated with changes in ACE activity. A large study of Serrano *et al*[43], considering 665 preeclamptic women and 1046 controls, did not find a significantly association of deletion form with preeclampsia. Li *et al*[44] in a Chinese population, investigated polymorphism of ACE gene and the polymorphism A1166C of angiotensin II receptor type 1 gene (AT1R). He found no significant differences in the frequency of genotypes of ACE gene and AT1R gene in preeclampsia and normal pregnancy, however preeclamptic women carrying the deletion form are more susceptible to developing renal dysfunction.

Another recent study investigated the association of both polymorphisms with the risk of preeclampsia[45] and it showed that the polymorphisms of Renin Angiotensin System could be associated with elevated oxidative stress, involved in preeclampsia development. Though is well know that the Renin Angiotensin System contribute to feto placental blood flow regulation, there are still no conclusive studies regarding association of genetic polymorphisms of this system and preeclampsia.

Also Nitric Oxide (NO) is an important regulator of vasodilatation and vascular remodelling and its production by nitric oxid synthase (eNOS) is known to be decreased in preeclampsia.

Firstly Hakli *et al*[46] evaluated the polymorphism Glu298Asp of eNOS gene in 132 preeclamptic women and 113 controls and they found a similar distribution of that polymorphism in both population.

A systematic review[38] on genes and preeclampsia, regarding the eNOS E298D polymorphism, concludes that this polymorphism does not seem to be related to a significantly increased risk of preeclampsia.

The production of vasoactive substances regulating the vascular tone is mediated by Estrogen Receptors α e β (ER α /β). Polymorphisms for these receptors have been reported associated with vascular disorders and pathogenesis of hypertension[47]. This study of Maruyama *et al*[47] considering the relationship between four SNPs (single nucleotide polymorphisms) in ER β and preeclampsia, found similar distribution of polymorphisms in preeclamptic women and control group. Another study[48] investigated two polymorphisms of the ER α gene (c.454 -397T>C and c.454 -351A>G) in 119 women with severe preeclampsia and 103 normotensive women and it found no association between severe preeclampsia and single gene polymorphism, however the presence of both polymorphisms (TT/AA genotypes) was significantly more frequent in severe preeclamptic patients than in normotensive population. However a study of Zhang[49] in Chinese population, conducted on 204 preeclamptic subjects and 236 normal women did not confirm these data, reporting a similar distribution in both groups of combined polymorphisms of ER α gene.

In recent years the attention is focused on binding of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) and their receptor Fms-like tyrosinkinase-1 receptor (sFlt-1) that stimulates placental vasculogenesis and angiogenesis: the interaction leads to a decreased circulating levels of PlGF and in preeclamptic women it is observed a increase in sFtl-1 and a corresponding decrease in PlGF. A recent meta-analysis[50] including 11 case-control studies analyzing 1069 preeclamptic women and 1315 normal pregnancies, concluded that VEGF polymorphisms +936C/T and -634G/C were associated with preeclampsia, and there was no evidence of the association between Only one study, to the best of our knowledge, regarding the polymorphisms in Flt-1 receptor has been published, based on the observation that a misregulation of Flt-1 results in over-expression of sFlt-1 and could contribute to physiopathology of preeclampsia. Kim *et al*[51] did not find significant difference in frequencies of the dinucleotide repeat polymorphism in preeclamptic women and in normotensive group.

**Genes implicated in oxidative stress:** It has been reported that the oxidative stress plays an important role in the aetiology of preeclampsia. Indeed in presence of imbalance between reactive oxygen species (ROS) production and antioxidant defence, the placental oxidative stress may stimulate syncytiothrophoblast apoptosis resulting in impaired placental function characteristic of preeclampsia[52].

In recent years the expression of OLR1 gene encoding for LOX-1 receptor (Low-density lipoprotein oxidized) was investigated in preeclamptic women. Indeed LOX-1, extensively studied for its role in myocardial ischemia, is a powerful mediator of endothelial dysfunction through generation of superoxide, induction of chemokine expression and inhibition of nitric oxide production leading to cell apoptosis[53]. An immunohistochemical study in preeclamptic placentas showed LOX-1 positive specimens in syncytiothrophoblast significantly upregulated comparing with normal placentas, confirming the elevated apoptotic activity of syncytiotrophoblast in preeclampsia[53].

Also the western blot examination of OLR-1 expression in syncytiothrophoblast had a higher expression in case of preeclampsia and other pregnancy diseases[54]. Within placental cells OLR1 is the main scavenger receptor responsible of up-take of LDL-ox. The high level of OLR1 expression is evidence of enhanced oxidative stress in preeclamptic placentas, in agreement with previous observations of elevated levels of serum lipid peroxides in preeclampsia[55,56].

Also polymorphisms in genes involved in the production of ROS or in the metabolism of these reactive species can lead to placental dysfunction.

Among anti-oxidant systems an important role is played by placental Glutatione S-transferase (GST) which contributes to placental detoxification. First Zusterzeel *et al*[57] reported that homozygous genotype GST 1b/1b was significantly more represented in preeclamptic women than in normotensive controls (OR 3.4) which could result in lower detoxification capacity.

Conversely Kim *et al*[58] in their study considering 214 normotensive pregnant women and 121 preeclamptic patients, showed that GST gene polymorphisms, as well as polymorphisms in the oxidative stress related genes, do not seem to be factors of susceptibility to preeclampsia.

Also Cytochrome P4501A1(CYP1A1) was related to preeclampsia, however no study did demonstrate the association between the single CYP4501A1 and preeclampsia[58].

Though single polymorphism don’t seem to increase susceptibility to gestational hypertensive disorders, however Zusterzeel *et al*[59], studying the simultaneous occurrence of severe genetic polymorphisms (GST, epoxyde hydrolase and CYP 1A1) in women developing preeclampsia, described a significant association between higher ROS production or lower detoxification pattern and preeclampia development.

Also polymorphisms of the gene encoding for Superoxide Dismutase (SOD) were investigated, SOD acting as cell protectors from superoxide radicals. Kim *et al*[59] reported no association with gene polymorphisms and susceptibility for preeclampsia. More recently two missense polymorphisms of extracellular SOD (arg213Gly and Ala40Thr) were investigated in 114 normotensive women and 159 preeclamptic patients and no significant differences were found, but it has been demonstrated that women carrying these polymorphisms do present a higher risk of severe preeclampsia complicated by fetal growth restriction[60]. Another recent study[61] in Romanian women described the genotype Val/Val significantly associated with preeclampsia and with a more clinical severity of the disease.

**Inherited thrombophilias:** The observation that women developing preeclampsia have subsequently a higher risk of thromboembolism, has often suggested the existence of a correlation between inherited thrombophilias and preeclampsia[62,63]. Also the occurrence of villous thrombosis is considered an important mechanism in the pathogenesis of preeclampsia. The condition of inherited thrombophilias is generated by specific polymorphisms in genes encoding for specific coagulation factors. These polymorphisms include the Factor V Leiden mutation (G1691G>A mutation Factor V), the methylentetraydrofolate reductase (MTHFR) (MTHFR 677C>T), the prothrombin mutation (G20210G>A) and the plasminogen activator inhibitor-1 mutant genotype (PAI-1 4G/4G>5G/5G).

First Dekker *et al*[64] in 1995 described an association between inherited thombophilias and severe preeclampsia. Since then, many studies have followed on the role of thrombophilic mutations in gestational hypertensive disorders, with contradictory results. In a review of year 2005 Cardewood *et al*[65] reports inconclusive results for the absence of large scale, randomised controlled studies. However he did underline a feasible association between placental troubles and Factor V Leiden. A large meta-analysis of Kosmas *et al*[66] focused on factor V Leiden, considering almost 3000 women, reports an odds ratio of 2.3, showing an important role of this polymorphism as a risk factor for preeclampsia. The same author reports[67] a moderately increased risk of preeclampsia in carriers of heterozygous and homozygous mutation of MTHFR 667 (OR 1.3). However a subsequent review of Pabinger[68] considering several interesting studies, reports no association between Factor V Leiden and prothrombin mutation (G20210G>A) versus hypertensive gestational disorders.

Also our study group analysed a link between inherited thrombophilias and preeclampsia, considering preeclamptic women and normal pregnant women and we had no evidence of association between preeclampsia and Factor V Leiden or prothrombin gene mutation[69]. Given the low positive predictive value of single thrombophilia in detection of preeclamptic risk, we conducted another study considering the association of double inherited thrombophilias and risk of adverse pregnancy outcomes. We had a slight but significant association between the combination of MTHFR C677T with Factor VIII and the combination of factor II and Factor V mutations and the occurrence of abruptio placentae, however we don’t find an increased incidence of adverse pregnancy outcomes in subjects with a combination of MTHFR C677T and Factor V Leiden or in patients with the simultaneous presence of Factor II mutation and PAI-1 (G5/G5)[70].

A recent review[71] regarding preeclampsia and inherited thrombophilias reports that mild preeclampsia is unlikely associated with thrombophilias, but severe and early-onset preeclampsia seem to be significantly related to inherited thrombophilias, and preeclamptic patients carrying gene mutations are at greater risk of developing more severe forms and sequelae.

In agreement with this findings, our study group highlighted that in preeclamptic patients with inherited thrombophilias a more severe involvement of kidney and a more severe damage in course of hypertensive gestational disease might occur[72].

It is therefore clear that contradictory results rise regarding association between thrombophilc gene mutations and preeclampsia and there aren’t consistent data to suggest a mandatory thrombophilic screening as predictive of preeclampsia.

***New biochemical markers***

In obstetrical practice a long-term objective is to identify ideal maternal biomarkers for preeclampsia, but it is very difficult because the “ideal marker” requires the coexistence of different characteristics: noninvasiveness, high sensitivity and specificity, high positive predictive value to predict disease prognosis. Currently we have a plethora of studies intended to identify an ideal biomarker, however differences in the studied populations, in the methodologies and in the results interpretations, make it difficult to perform a systematic analysis of all the markers (Table 2). Therefore, in this review we will consider only markers proposed more recently as potential new biomarkers.

Research of these new emerging biomarkers arises from the new model of pathogenesis of preeclampsia which places the focus not longer on vasocontrictive phenomenon but on the angiogenesis process[81].

Among proangiogenic factors there are vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), among antiangiogenic factor there are soluble endoglin (sEng) and soluble fms-like tyrosine kinase 1 receptor(sFlt-1).

Cells expressing VEGF are located near fenestrated endothelia and the inhibition of VEGF leads to pathological conditions in many organs with fenestrated endothelia (*e.g.,* liver, kidney, choroid plexus *etc.*) as observed in severe preeclampsia. PlGF is expressed mainly by placental cells and its levels increase from the second to third trimester. Both VEGF and PlGF bind VEGF receptor family, named Flt-1 and KDR. PlGF binds more actively Flt-1, while VEGF binds KDR. It has been suggested that sFlt-1 acts modulating VEGF availability[82].

This evidence confirms the antiagiogenic role of soluble form of VEGF-PlGF receptor sFlt-1.

sFlt-1binds these angiogenic factors and inhibits their vasodilatory effect. Other anti-angiogenic factor is sEng. In animal studies it allows the formation of endothelial tube and increases capillary permeability and could be responsible of hypertension, nephrotic syndrome and liver dysfunction during preeclampsia[83].

A recent review reported significant changes in levels of sFlt-1, PlGF and sEng in preeclamptic patients, but with a different time course, earliest in the first trimester for PlGF and later for sFlt-1 and sEng.

Levine*et al*[84,85] in two studies of 2004 and 2006, demonstrated that level of sFlt-1 increased 5 wk before the onset of clinical disease and parallel levels of PlGF and VEGF decrease, due to the binding by sFlt-1 while level of sEng increases 2-3 mo before clinical disease.

More recently the level of placental growth factor (PIGF) is evaluated in pregnancy complicated by hypertension disease[86] and it has been found that a positive PIGF test can predict delivery before 37 wk in over 90% of pregnant women with hypertensive disease. Therefore a low level of PIGF could be used before 35 wk, in hypertensive women, to evaluate the risk of pregnancy complications. Also sEng level seems to be a prognostic, and its level appears correlated with severe preeclampsia or eclampsia[87]. Despite this evidence, there aren’t yet conclusive data on their diagnostic capability, on the cut-off of normality and on the time or the strategy to measure these markers.

Regarding diagnostic capability, a recent extensive study conducted on 2200 patients reported for PlGF and sFlt-1 at first trimester, a sensitivity of 55% and 57% respectively and a specificity of 43% and 40% respectively[88] and this result doesn’t improve later in pregnancy. It is evident that the predictive positive value is too low to use this marker at first trimester as screening of preeclampsia. Other strategies in measuring the angiogenic factors have been proposed: a longitudinal evaluation and ratio between two factors.

Indeed in preeclamptic women it has been demonstrated an increase from first to second trimester of sFlt-1, sEng and PlGF[89], on the other hand several studies, based on the observation that levels of PlGF and sFlt-1 are altered together in preeclampsia, proposed a ratio between sFlt-1 and PlGF (sFlt-1:PlGF)[90] and between PlGF and sEng (PlGF:sEng)[91] reporting an important improvement in sensitivity (88.5% and 100% respectively) and in specificity (88.5% and 98% respectively). Despite these promising results, larger studies are needed to confirm these finding.

Our brief review regarding the possibility of an early screening for preeclampsia, analysed the most recent literature and highlighted the lack of a single certified method able to predict the risk. However, despite the complexity of clinical and physiopathological behaviour of preeclampsia, it is possible that in the future the combination of several tests will allow us to predict women at risk of preeclampsia.

One point needs to be underlined: we started from ultrasonic evaluations (Uterine arteries Doppler US) and in a relatively short period, we overcome to a supersonic era, in which more promising and accurate tests seem to came from laboratory.

**REFERENCES**

1 **Cantwell R**, Clutton-Brock T, Cooper G, Dawson A, Drife J, Garrod D, Harper A, Hulbert D, Lucas S, McClure J, Millward-Sadler H, Neilson J, Nelson-Piercy C, Norman J, O'Herlihy C, Oates M, Shakespeare J, de Swiet M, Williamson C, Beale V, Knight M, Lennox C, Miller A, Parmar D, Rogers J, Springett A. Saving Mothers' Lives: Reviewing maternal deaths to make motherhood safer: 2006-2008. The Eighth Report of the Confidential Enquiries into Maternal Deaths in the United Kingdom. *BJOG* 2011; **118** Suppl 1: 1-203 [PMID: 21356004]

2 Centre for Maternal and Child Enquires (CMACE) Perinatal mortality 2009: United Kingdom. CMACE, London, 2011.

3 **Brown MA**, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy* 2001; **20**: IX-XIV [PMID: 12044323 DOI: 10.3109/10641950109152635]

4 **Brown MA**, Hague WM, Higgins J, Lowe S, McCowan L, Oats J, Peek MJ, Rowan JA, Walters BN. The detection, investigation and management of hypertension in pregnancy: executive summary. *Aust N Z J Obstet Gynaecol* 2000; **40**: 133-138 [PMID: 10925899 DOI: 10.1111/j.1479-828X.2000.tb01136.x]

5 Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. *Am J Obstet Gynecol* 2000; **183**: S1-S22 [PMID: 10920346 DOI: 10.1016/S0002-9378(00)99785-0]

6 **Lindheimer MD**, Taler SJ, Cunningham FG. ASH position paper: hypertension in pregnancy. *J Clin Hypertens (Greenwich)* 2009; **11**: 214-225 [PMID: 19614806 DOI: 10.1111/j.1751-7176.2009.00085.x]

7 National Institute for health and clinical excellence. Hypertension in pregnancy. The management of hypertensive disorders during pregnancy. NICE clinical guideline 107. Last modified January 2011.

8 -Royal College of Obstetricians and Gynaecologists. The management of severe preeclampsia. RCOG guideline N°10, reviewed 2010.

9 **McCowan LM**, Ritchie K, Mo LY, Bascom PA, Sherret H. Uterine artery flow velocity waveforms in normal and growth-retarded pregnancies. *Am J Obstet Gynecol* 1988; **158**: 499-504 [PMID: 2964782 DOI: 10.1016/0002-9378(88)90013-0]

10 **Campbell S**, Pearce JM, Hackett G, Cohen-Overbeek T, Hernandez C. Qualitative assessment of uteroplacental blood flow: early screening test for high-risk pregnancies. *Obstet Gynecol* 1986; **68**: 649-653 [PMID: 2945132]

11 **Valensise H**, Bezzeccheri V, Rizzo G, Tranquilli AL, Garzetti GG, Romanini C. Doppler velocimetry of the uterine artery as a screening test for gestational hypertension. *Ultrasound Obstet Gynecol* 1993; **3**: 18-22 [PMID: 12796896 DOI: 10.1046/j.1469-0705.1993.03010018.x]

12 **Jacobson SL**, Imhof R, Manning N, Mannion V, Little D, Rey E, Redman C. The value of Doppler assessment of the uteroplacental circulation in predicting preeclampsia or intrauterine growth retardation. *Am J Obstet Gynecol* 1990; **162**: 110-114 [PMID: 2405672 DOI: 10.1016/0002-9378(90)90832-R]

13 **Arduini D**, Rizzo G, Romanini C, Mancuso S. Utero-placental blood flow velocity waveforms as predictors of pregnancy-induced hypertension. *Eur J Obstet Gynecol Reprod Biol* 1987; **26**: 335-341 [PMID: 2961632 DOI: 10.1016/0028-2243(87)90131-6]

14 **Zimmermann P**, Eiriö V, Koskinen J, Kujansuu E, Ranta T. Doppler assessment of the uterine and uteroplacental circulation in the second trimester in pregnancies at high risk for pre-eclampsia and/or intrauterine growth retardation: comparison and correlation between different Doppler parameters. *Ultrasound Obstet Gynecol* 1997; **9**: 330-338 [PMID: 9201877 DOI: 10.1046/j.1469-0705.1997.09050330.x]

15 **Bower S**, Schuchter K, Campbell S. Doppler ultrasound screening as part of routine antenatal scanning: prediction of pre-eclampsia and intrauterine growth retardation. *Br J Obstet Gynaecol* 1993; **100**: 989-994 [PMID: 8251470]

16 **Chan FY**, Pun TC, Lam C, Khoo J, Lee CP, Lam YH. Pregnancy screening by uterine artery Doppler velocimetry--which criterion performs best? *Obstet Gynecol* 1995; **85**: 596-602 [PMID: 7898840 DOI: 10.1016/0029-7844(95)00006-D]

17 **North RA**, Ferrier C, Long D, Townend K, Kincaid-Smith P. Uterine artery Doppler flow velocity waveforms in the second trimester for the prediction of preeclampsia and fetal growth retardation. *Obstet Gynecol* 1994; **83**: 378-386 [PMID: 8127529]

18 **Steel SA**, Pearce JM, McParland P, Chamberlain GV. Early Doppler ultrasound screening in prediction of hypertensive disorders of pregnancy. *Lancet* 1990; **335**: 1548-1551 [PMID: 1972486 DOI: 10.1016/0140-6736(90)91376-L]

19 **Bower S**, Bewley S, Campbell S. Improved prediction of preeclampsia by two-stage screening of uterine arteries using the early diastolic notch and color Doppler imaging. *Obstet Gynecol* 1993; **82**: 78-83 [PMID: 8515930]

20 **Caruso A**, Caforio L, Testa AC, Ferrazzani S, Mastromarino C, Mancuso S. Chronic hypertension in pregnancy: color Doppler investigation of uterine arteries as a predictive test for superimposed preeclampsia and adverse perinatal outcome. *J Perinat Med* 1996; **24**: 141-153 [PMID: 8773940 DOI: 10.1515/jpme.1996.24.2.141]

21 **Parretti E**, Mealli F, Magrini A, Cioni R, Mecacci F, La Torre P, Periti E, Scarselli G, Mello G. Cross-sectional and longitudinal evaluation of uterine artery Doppler velocimetry for the prediction of pre-eclampsia in normotensive women with specific risk factors. *Ultrasound Obstet Gynecol* 2003; **22**: 160-165 [PMID: 12905511 DOI: 10.1002/uog.194]

22 **Valensise H**, Tranquilli AL, Arduini D, Garzetti GG, Romanini C. Screening pregnant women at 22-24 weeks for gestational hypertension or intrauterine growth retardation by Doppler ultrasound followed by 24-h blood pressure recording. *Hypertension Pregn* 1995: **14**: 351-60. doi: 10.3109/10641959509015681

23 **Ashour AM**, Lieberman ES, Haug LE, Repke JT. The value of elevated second-trimester beta-human chorionic gonadotropin in predicting development of preeclampsia. *Am J Obstet Gynecol* 1997; **176**: 438-442 [PMID: 9065195 DOI: 10.1016/S0002-9378(97)70512-X]

24 **Elsandabesee D**, Srinivas M, Kodakkattil S. The clinical value of combining maternal serum screening and uterine artery Doppler in prediction of adverse pregnancy outcome. *J Obstet Gynaecol* 2006; **26**: 115-117 [PMID: 16483965 DOI: 10.1080/01443610500443279]

25 **Chafetz I**, Kuhnreich I, Sammar M, Tal Y, Gibor Y, Meiri H, Cuckle H, Wolf M. First-trimester placental protein 13 screening for preeclampsia and intrauterine growth restriction. *Am J Obstet Gynecol* 2007; **197**: 35.e1-35.e7 [PMID: 17618748 DOI: 10.1016/j.ajog.2007.02.025]

26 **Romero R**, Kusanovic JP, Than NG, Erez O, Gotsch F, Espinoza J, Edwin S, Chefetz I, Gomez R, Nien JK, Sammar M, Pineles B, Hassan SS, Meiri H, Tal Y, Kuhnreich I, Papp Z, Cuckle HS. First-trimester maternal serum PP13 in the risk assessment for preeclampsia. *Am J Obstet Gynecol* 2008; **199**: 122.e1-122.e11 [PMID: 18539259]

27 **Stamatopoulou A**, Cowans NJ, Matwejew E, von Kaisenberg C, Spencer K. Placental protein-13 and pregnancy-associated plasma protein-A as first trimester screening markers for hypertensive disorders and small for gestational age outcomes. *Hypertens Pregnancy* 2011; **30**: 384-395 [PMID: 20701472]

28 **Akolekar R**, Syngelaki A, Beta J, Kocylowski R, Nicolaides KH. Maternal serum placental protein 13 at 11-13 weeks of gestation in preeclampsia. *Prenat Diagn* 2009; **29**: 1103-1108 [PMID: 19777530 DOI: 10.1002/pd.2375]

29 **Giguère Y**, Charland M, Bujold E, Bernard N, Grenier S, Rousseau F, Lafond J, Légaré F, Forest JC. Combining biochemical and ultrasonographic markers in predicting preeclampsia: a systematic review. *Clin Chem* 2010; **56**: 361-375 [PMID: 20044446 DOI: 10.1373/clinchem.2009.134080]

30 **Valensise H**, Novelli GP, Vasapollo B, Borzi M, Arduini D, Galante A, Romanini C. Maternal cardiac systolic and diastolic function: relationship with uteroplacental resistances. A Doppler and echocardiographic longitudinal study. *Ultrasound Obstet Gynecol* 2000; **15**: 487-497 [PMID: 11005116 DOI: 10.1046/j.1469-0705.2000.00135.x]

31 **Valensise H**, Andreoli A, Lello S, Magnani F, Romanini C, De Lorenzo A. Multifrequency bioelectrical impedance analysis in women with a normal and hypertensive pregnancy. *Am J Clin Nutr* 2000; **72**: 780-783 [PMID: 10966899]

32 **Nisell H**, Lunell NO, Linde B. Maternal hemodynamics and impaired fetal growth in pregnancy-induced hypertension. *Obstet Gynecol* 1988; **71**: 163-166 [PMID: 3336550]

33 **Duvekot JJ**, Cheriex EC, Pieters FA, Peeters LL. Severely impaired fetal growth is preceded by maternal hemodynamic maladaptation in very early pregnancy. *Acta Obstet Gynecol Scand* 1995; **74**: 693-697 [PMID: 7572102 DOI: 10.3109/00016349509021176]

34 **Novelli GP**, Vasapollo B, Gagliardi G, Tiralongo GM, Pisani I, Manfellotto D, Giannini L, Valensise H. Left ventricular midwall mechanics at 24 weeks' gestation in high-risk normotensive pregnant women: relationship to placenta-related complications of pregnancy. *Ultrasound Obstet Gynecol* 2012; **39**: 430-437 [PMID: 22411543 DOI: 10.1002/uog.10089]

35 **Valensise H**, Novelli GP, Vasapollo B, Di Ruzza G, Romanini ME, Marchei M, Larciprete G, Manfellotto D, Romanini C, Galante A. Maternal diastolic dysfunction and left ventricular geometry in gestational hypertension. *Hypertension* 2001; **37**: 1209-1215 [PMID: 11358930 DOI: 10.1161/01.HYP.37.5.1209]

36 **Valensise H**, Vasapollo B, Novelli GP, Larciprete G, Romanini ME, Arduini D, Galante A, Romanini C. Maternal diastolic function in asymptomatic pregnant women with bilateral notching of the uterine artery waveform at 24 weeks' gestation: a pilot study. *Ultrasound Obstet Gynecol* 2001; **18**: 450-455 [PMID: 11844163 DOI: 10.1046/j.0960-7692.2001.00576.x]

37 **Vasapollo B**, Novelli GP, Valensise H. Total vascular resistance and left ventricular morphology as screening tools for complications in pregnancy. *Hypertension* 2008; **51**: 1020-1026 [PMID: 18259001 DOI: 10.1161/HYPERTENSIONAHA.107.105858]

38 **Mütze S**, Rudnik-Schöneborn S, Zerres K, Rath W. Genes and the preeclampsia syndrome. *J Perinat Med* 2008; **36**: 38-58 [PMID: 18184097]

39 **Rajakumar A**, Chu T, Handley DE, Bunce KD, Burke B, Hubel CA, Jeyabalan A, Peters DG. Maternal gene expression profiling during pregnancy and preeclampsia in human peripheral blood mononuclear cells. *Placenta* 2011; **32**: 70-78 [PMID: 21075447 DOI: 10.1016/j.placenta.2010.10.004]

40 **Barden AE**, Herbison CE, Beilin LJ, Michael CA, Walters BN, Van Bockxmeer FM. Association between the endothelin-1 gene Lys198Asn polymorphism blood pressure and plasma endothelin-1 levels in normal and pre-eclamptic pregnancy. *J Hypertens* 2001; **19**: 1775-1782 [PMID: 11593097 DOI: 10.1097/00004872-200110000-00011]

41 **Seremak-Mrozikiewicz A**, Barlik M, Perlik M, Kurzawińska G, Drews K. [Genetic variability of endothelin-1 system in gestational hypertension and preeclampsia]. *Ginekol Pol* 2011; **82**: 363-370 [PMID: 21851036]

42 **Lisi V**, Paternoster DM, Stecca A, Micciché F, Fantinato S, Leon A, Damante G, Fabbro D, Clementi M. Investigation of endothelin-1 type A receptor gene polymorphism (-231 G & gt; A) in preeclampsia susceptibility. *J Matern Fetal Neonatal Med* 2007; **20**: 145-149 [PMID: 17437213 DOI: 10.1080/14767050601127797]

43 **Serrano NC**, Díaz LA, Páez MC, Mesa CM, Cifuentes R, Monterrosa A, González A, Smeeth L, Hingorani AD, Casas JP. Angiotensin-converting enzyme I/D polymorphism and preeclampsia risk: evidence of small-study bias. *PLoS Med* 2006; **3**: e520 [PMID: 17194198]

44 **Li H**, Ma Y, Fu Q, Wang L. Angiotensin-converting enzyme insertion/deletion (ACE I/D) and angiotensin II type 1 receptor (AT1R) gene polymorphism and its association with preeclampsia in Chinese women. *Hypertens Pregnancy* 2007; **26**: 293-301 [PMID: 17710578]

45 **Rahimi Z**, Rahimi Z, Mozafari H, Parsian A. Preeclampsia and angiotensin converting enzyme (ACE) I/D and angiotensin II type-1 receptor (AT1R) A1166C polymorphisms: association with ACE I/D polymorphism. *J Renin Angiotensin Aldosterone Syst* 2013; **14**: 174-180 [PMID: 22719026 DOI: 10.1177/1470320312448950]

46 **Häkli T**, Romppanen EL, Hiltunen M, Helisalmi S, Punnonen K, Heinonen S. Endothelial nitric oxide synthase polymorphism in preeclampsia. *J Soc Gynecol Investig* 2003; **10**: 154-157 [PMID: 12699878 DOI: 10.1016/S1071-5576(03)00003-0]

47 **Maruyama A**, Nakayama T, Sato N, Mizutani Y, Furuya K, Yamamoto T. Association study using single nucleotide polymorphisms in the estrogen receptor beta (ESR2) gene for preeclampsia. *Hypertens Res* 2004; **27**: 903-909 [PMID: 15894829 DOI: 10.1291/hypres.27.903]

48 **Molvarec A**, Vér A, Fekete A, Rosta K, Derzbach L, Derzsy Z, Karádi I, Rigó J. Association between estrogen receptor alpha (ESR1) gene polymorphisms and severe preeclampsia. *Hypertens Res* 2007; **30**: 205-211 [PMID: 17510501 DOI: 10.1291/hypres.30.205]

49 **Zhang J**, Bai H, Liu X, Fan P, Liu R, Huang Y, Wang X, He G, Liu Y, Liu B. Genotype distribution of estrogen receptor alpha polymorphisms in pregnant women from healthy and preeclampsia populations and its relation to blood pressure levels. *Clin Chem Lab Med* 2009; **47**: 391-397 [PMID: 19284296 DOI: 10.1515/CCLM.2009.096]

50 **Cheng D**, Hao Y, Zhou W, Ma Y. Vascular endothelial growth factor +936C/T, -634G/C, -2578C/A, and -1154G/A polymorphisms with risk of preeclampsia: a meta-analysis. *PLoS One* 2013; **8**: e78173 [PMID: 24223772 DOI: 10.1371/journal.pone.0078173]

51 **Kim SY**, Lim JH, Yang JH, Kim MY, Han JY, Ahn HK, Choi JS, Park SY, Kim MJ, Ryu HM. Dinucleotide repeat polymorphism in Fms-like tyrosine kinase-1 (Flt-1) gene is not associated with preeclampsia. *BMC Med Genet* 2008; **9**: 68 [PMID: 18631405 DOI: 10.1186/1471-2350-9-68]

52 **Sikkema JM**, van Rijn BB, Franx A, Bruinse HW, de Roos R, Stroes ES, van Faassen EE. Placental superoxide is increased in pre-eclampsia. *Placenta* 2001; **22**: 304-308 [PMID: 11286565 DOI: 10.1053/plac.2001.0629]

53 **Lee H**, Park H, Kim YJ, Kim HJ, Ahn YM, Park B, Park JH, Lee BE. Expression of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) in human preeclamptic placenta: possible implications in the process of trophoblast apoptosis. *Placenta* ; **26**: 226-233 [PMID: 15708124 DOI: 10.1016/j.placenta.2004.05.012]

54 **Ethier-Chiasson M**, Forest JC, Giguère Y, Masse A, Marseille-Tremblay C, Lévy E, Lafond J. Modulation of placental protein expression of OLR1: implication in pregnancy-related disorders or pathologies. *Reproduction* 2008; **136**: 491-502 [PMID: 18599643 DOI: 10.1530/REP-08-0082]

55 **Gratacós E**, Casals E, Deulofeu R, Cararach V, Alonso PL, Fortuny A. Lipid peroxide and vitamin E patterns in pregnant women with different types of hypertension in pregnancy. *Am J Obstet Gynecol* 1998; **178**: 1072-1076 [PMID: 9609586 DOI: 10.1016/S0002-9378(98)70550-2]

56 **Serdar Z**, Gür E, Develioğlu O, Colakoğullari M, Dirican M. Placental and decidual lipid peroxidation and antioxidant defenses in preeclampsia. Lipid peroxidation in preeclampsia. *Pathophysiology* 2002; **9**: 21 [PMID: 12385961 DOI: 10.1016/S0928-4680(02)00052-4]

57 **Zusterzeel PL**, Visser W, Peters WH, Merkus HW, Nelen WL, Steegers EA. Polymorphism in the glutathione S-transferase P1 gene and risk for preeclampsia. *Obstet Gynecol* 2000; **96**: 50-54 [PMID: 10862841 DOI: 10.1016/S0029-7844(00)00845-0]

58 **Kim YJ**, Park HS, Park MH, Suh SH, Pang MG. Oxidative stress-related gene polymorphism and the risk of preeclampsia. *Eur J Obstet Gynecol Reprod Biol* 2005; **119**: 42-46 [PMID: 15734083 DOI: 10.1016/j.ejogrb.2004.06.009]

59 **Zusterzeel PL**, Peters WH, Burton GJ, Visser W, Roelofs HM, Steegers EA. Susceptibility to pre-eclampsia is associated with multiple genetic polymorphisms in maternal biotransformation enzymes. *Gynecol Obstet Invest* 2007; **63**: 209-213 [PMID: 17167268 DOI: 10.1159/000097987]

60 **Rosta K**, Molvarec A, Enzsöly A, Nagy B, Rónai Z, Fekete A, Sasvári-Székely M, Rigó J, Vér A. Association of extracellular superoxide dismutase (SOD3) Ala40Thr gene polymorphism with pre-eclampsia complicated by severe fetal growth restriction. *Eur J Obstet Gynecol Reprod Biol* 2009; **142**: 134-138 [PMID: 19108943 DOI: 10.1016/j.ejogrb.2008.10.014]

61 **Procopciuc LM**, Caracostea G, Nemeti G, Drugan C, Olteanu I, Stamatian F. The Ala-9Val (Mn-SOD) and Arg213Gly (EC-SOD) polymorphisms in the pathogenesis of preeclampsia in Romanian women: association with the severity and outcome of preeclampsia. *J Matern Fetal Neonatal Med* 2012; **25**: 895-900 [PMID: 22432908 DOI: 10.3109/14767058.2011.599078]

62 **van Walraven C**, Mamdani M, Cohn A, Katib Y, Walker M, Rodger MA. Risk of subsequent thromboembolism for patients with pre-eclampsia. *BMJ* 2003; **326**: 791-792 [PMID: 12689975 DOI: 10.1136/bmj.326.7393.791]

63 **Brenner B**, Lanir N, Thaler I. HELLP syndrome associated with factor V R506Q mutation. *Br J Haematol* 1996; **92**: 999-1001 [PMID: 8616100 DOI: 10.1046/j.1365-2141.1996.410947.x]

64 **Dekker GA**, de Vries JI, Doelitzsch PM, Huijgens PC, von Blomberg BM, Jakobs C, van Geijn HP. Underlying disorders associated with severe early-onset preeclampsia. *Am J Obstet Gynecol* 1995; **173**: 1042-1048 [PMID: 7485291 DOI: 10.1016/0002-9378(95)91324-6]

65 **Calderwood CJ**, Greer IA. The role of factor V Leiden in maternal health and the outcome of pregnancy. *Curr Drug Targets* 2005; **6**: 567-576 [PMID: 16026277 DOI: 10.2174/1389450054546024]

66 **Kosmas IP**, Tatsioni A, Ioannidis JP. Association of Leiden mutation in factor V gene with hypertension in pregnancy and pre-eclampsia: a meta-analysis. *J Hypertens* 2003; **21**: 1221-1228 [PMID: 12817161 DOI: 10.1097/00004872-200307000-00002]

67 **Kosmas IP**, Tatsioni A, Ioannidis JP. Association of C677T polymorphism in the methylenetetrahydrofolate reductase gene with hypertension in pregnancy and pre-eclampsia: a meta-analysis. *J Hypertens* 2004; **22**: 1655-1662 [PMID: 15311088 DOI: 10.1097/00004872-200409000-00004]

68 **Pabinger I**, Vormittag R. Thrombophilia and pregnancy outcomes. *J Thromb Haemost* 2005; **3**: 1603-1610 [PMID: 16102025 DOI: 10.1111/j.1538-7836.2005.01417.x]

69 **Larciprete G**, Gioia S, Angelucci PA, Brosio F, Barbati G, Angelucci GP, Frigo MG, Baiocco F, Romanini ME, Arduini D, Cirese E. Single inherited thrombophilias and adverse pregnancy outcomes. *J Obstet Gynaecol Res* 2007; **33**: 423-430 [PMID: 17688607 DOI: 10.1111/j.1447-0756.2007.00550.x]

70 **Larciprete G**, Rossi F, Deaibess T, Brienza L, Barbati G, Romanini E, Gioia S, Cirese E. Double inherited thrombophilias and adverse pregnancy outcomes: fashion or science? *J Obstet Gynaecol Res* 2010; **36**: 996-1002 [PMID: 20868443]

71 **Rath W**. Pre-eclampsia and inherited thrombophilia: a reappraisal. *Semin Thromb Hemost* 2011; **37**: 118-124 [PMID: 21370211 DOI: 10.1055/s-0030-1270337]

72 **Giovanni L**, Maria LG, Mauro R, Carlotta M, Federica R, Fabrizio P, Sheba J, Giuseppe DP, Alessandro B, Elio C, Herbert V. Thrombophilia and damage of kidney during pregnancy. *J Prenat Med* 2011; **5**: 78-82 [PMID: 22905298]

73 **Jauniaux E**, Gulbis B, Tunkel S, Ramsay B, Campbell S, Meuris S. Maternal serum testing for alpha-fetoprotein and human chorionic gonadotropin in high-risk pregnancies. *Prenat Diagn* 1996; **16**: 1129-1135 [PMID: 8994249]

74 **Merviel P**, Müller F, Guibourdenche J, Berkane N, Gaudet R, Bréart G, Uzan S. Correlations between serum assays of human chorionic gonadotrophin (hCG) and human placental lactogen (hPL) and pre-eclampsia or intrauterine growth restriction (IUGR) among nulliparas younger than 38 years. *Eur J Obstet Gynecol Reprod Biol* 2001; **95**: 59-67 [PMID: 11267722 DOI: 10.1016/S0301-2115(00)00370-5]

75 **Spencer K**, Cowans NJ, Nicolaides KH. Maternal serum inhibin-A and activin-A levels in the first trimester of pregnancies developing pre-eclampsia. *Ultrasound Obstet Gynecol* 2008; **32**: 622-626 [PMID: 18816493 DOI: 10.1002/uog.6212]

76 **Florio P**, Reis FM, Pezzani I, Luisi S, Severi FM, Petraglia F. The addition of activin A and inhibin A measurement to uterine artery Doppler velocimetry to improve the early prediction of pre-eclampsia. *Ultrasound Obstet Gynecol* 2003; **21**: 165-169 [PMID: 12601840 DOI: 10.1002/uog.29]

77 **Nicolaides KH**, Bindra R, Turan OM, Chefetz I, Sammar M, Meiri H, Tal J, Cuckle HS. A novel approach to first-trimester screening for early pre-eclampsia combining serum PP-13 and Doppler ultrasound. *Ultrasound Obstet Gynecol* 2006; **27**: 13-17 [PMID: 16374755]

78 **Kuo PL**, Lin CC, Lin YH, Guo HR. Placental sonolucency and pregnancy outcome in women with elevated second trimester serum alpha-fetoprotein levels. *J Formos Med Assoc* 2003; **102**: 319-325 [PMID: 12874670]

79 **Poon LC**, Maiz N, Valencia C, Plasencia W, Nicolaides KH. First-trimester maternal serum pregnancy-associated plasma protein-A and pre-eclampsia. *Ultrasound Obstet Gynecol* 2009; **33**: 23-33 [PMID: 19090499 DOI: 10.1002/uog.6280]

80 **Spencer K**, Yu CK, Cowans NJ, Otigbah C, Nicolaides KH. Prediction of pregnancy complications by first-trimester maternal serum PAPP-A and free beta-hCG and with second-trimester uterine artery Doppler. *Prenat Diagn* 2005; **25**: 949-953 [PMID: 16086443 DOI: 10.1002/pd.1251]

81 **Naljayan MV**, Karumanchi SA. New developments in the pathogenesis of preeclampsia. *Adv Chronic Kidney Dis* 2013; **20**: 265-270 [PMID: 23928392 DOI: 10.1053/j.ackd.2013.02.003]

82 **Chappell JC**, Taylor SM, Ferrara N, Bautch VL. Local guidance of emerging vessel sprouts requires soluble Flt-1. *Dev Cell* 2009; **17**: 377-386 [PMID: 19758562 DOI: 10.1016/j.devcel.2009.07.011]

83 **Venkatesha S**, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, Bdolah Y, Lim KH, Yuan HT, Libermann TA, Stillman IE, Roberts D, D'Amore PA, Epstein FH, Sellke FW, Romero R, Sukhatme VP, Letarte M, Karumanchi SA. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med* 2006; **12**: 642-649 [PMID: 16751767 DOI: 10.1038/nm1429]

84 **Levine RJ**, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 2004; **350**: 672-683 [PMID: 14764923 DOI: 10.1056/NEJMoa031884]

85 **Levine RJ**, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R, Karumanchi SA. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med* 2006; **355**: 992-1005 [PMID: 16957146 DOI: 10.1056/NEJMoa055352]

86 **Molvarec A**, Gullai N, Stenczer B, Fügedi G, Nagy B, Rigó J. Comparison of placental growth factor and fetal flow Doppler ultrasonography to identify fetal adverse outcomes in women with hypertensive disorders of pregnancy: an observational study. *BMC Pregnancy Childbirth* 2013; **13**: 161 [PMID: 23937721 DOI: 10.1186/1471-2393-13-161]

87 **Vaisbuch E**, Whitty JE, Hassan SS, Romero R, Kusanovic JP, Cotton DB, Sorokin Y, Karumanchi SA. Circulating angiogenic and antiangiogenic factors in women with eclampsia. *Am J Obstet Gynecol* 2011; **204**: 152.e1-152.e9 [PMID: 21062661]

88 **McElrath TF**, Lim KH, Pare E, Rich-Edwards J, Pucci D, Troisi R, Parry S. Longitudinal evaluation of predictive value for preeclampsia of circulating angiogenic factors through pregnancy. *Am J Obstet Gynecol* 2012; **207**: 407.e1-407.e7 [PMID: 22981320]

89 **Moore Simas TA**, Crawford SL, Solitro MJ, Frost SC, Meyer BA, Maynard SE. Angiogenic factors for the prediction of preeclampsia in high-risk women. *Am J Obstet Gynecol* 2007; **197**: 244.e1-244.e8 [PMID: 17826405 DOI: 10.1016/j.ajog.2007.06.030]

90 **De Vivo A**, Baviera G, Giordano D, Todarello G, Corrado F, D'anna R. Endoglin, PlGF and sFlt-1 as markers for predicting pre-eclampsia. *Acta Obstet Gynecol Scand* 2008; **87**: 837-842 [PMID: 18607829]

91 **Kusanovic JP**, Romero R, Chaiworapongsa T, Erez O, Mittal P, Vaisbuch E, Mazaki-Tovi S, Gotsch F, Edwin SS, Gomez R, Yeo L, Conde-Agudelo A, Hassan SS. A prospective cohort study of the value of maternal plasma concentrations of angiogenic and anti-angiogenic factors in early pregnancy and midtrimester in the identification of patients destined to develop preeclampsia. *J Matern Fetal Neonatal Med* 2009; **22**: 1021-1038 [PMID: 19900040 DOI: 10.3109/14767050902994754]

**P-Reviewers:** Mimura K, Tsikouras P **S-Editor:** Wen LL  **L-Editor:**  **E-Editor:**

**Table 1 Uterine artery Doppler studies for the prediction of preeclampsia**

|  |  |  |  |
| --- | --- | --- | --- |
| **Ref.** | **Week of evaluation** | **Sensitivity** | **Specificity** |
| Campbell *et al*[10]  | 16-18 | 68% | 69% |
| Valensise *et al*[11] | 22 | 74% | 97.5% |
| Jacobson *et al*[12] | 24 | 44% | 73% |
| Arduini *et al*[13] | 18-20 | 64% | 94% |
| Ziemmermann *et al*[14] | 21-24 | 56% | 83% |
| Bower *et al*[15] | 18-22 | 75% | 86% |
| Chan *et al*[16] | 20 | 27% | 97% |
| North *et al*[17] | 19-24 | 27% | 90% |

**Table 2 Biochemical markers predicting preeclampsia**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Markers** | **Ref.** | **Age of testing** | **Sensitivity** | **Specificity** |
| hCG | Jauniaux *et al*[73]Merviel *et al*[74] | 2nd trimester2nd trimester | 72.7%54.5% | 90%93.5% |
| Inhibin A | Spencer *et al*[75]Florio *et al*[76] | 1st or 2nd  trimester2nd trimester | 68%38.9% | 95%92.5% |
| PP-13 | Nicholaides *et al*[77] | 1st trimester | 90% | 90% |
| AFP | Jauniaux *et al*[73]Kuo *et al*[78] | 2nd  trimester2nd  trimester  | 72.7%61.5% |  70%47.3% |
| Activin A | Florio *et al*[76]Spencer *et al*[75] | 2nd  trimester2nd trimester | 61.1%63% | 77.5%95% |
| PAPP-A | Poon *et al*[79]Spencer *et al*[80] | 1st trimester1st trimester | 20.5%62.1% | 95%95% |