



Prediction of Preeclampsia by Uterine Artery Doppler Examination and Placental Growth Factor, Endoglin and Pregnancy-associated Plasma Protein Levels in Maternal Serum at 11-13+6 Pregnancy Week

İD Şule Yıldırım Köpük¹, İD Yiğit Çakıroğlu², İD Yasin Ceylan³, İD Mustafa Baki Çekmen⁴, İD Gülseren Yücesoy²

¹Ümraniye Training and Research Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey

²Kocaeli University Faculty of Medicine, Department of Obstetrics and Gynecology, Kocaeli, Turkey

³Mardin Kızıltepe State Hospital, Clinic of Obstetrics and Gynecology, Mardin, Turkey

⁴Medeniyet University Faculty of Medicine, Department of Biochemistry, İstanbul, Turkey

Abstract

Objective: We aimed to evaluate the prediction of preeclampsia by maternal serum pregnancy-associated plasma protein A (PAPP-A), placental growth factor (PIGF), sEndoglin (sEng) and uterine artery Doppler examination.

Methods: One hundred and ninety-three singleton pregnant women admitted to outpatient clinic at 11-13+6 weeks for first trimester combined screening test were included in the study. Maternal history was taken, and uterine artery Doppler examination and serum biomarker screening (PAPP-A, PIGF, sEndoglin) were conducted. The follow-up results of pregnancy were recorded. The study cohort was divided into two groups as “control” and “preeclampsia”. Mann-Whitney U and chi-square tests were used for comparison of independent variables. Sensitivity and specificity for the predictive values of the significant parameters were calculated from ROC curves.

Results: Of 193 women, 168 women (87%) were in the control group (group 1) and 25 women (12.9%) who developed preeclampsia were defined in the preeclampsia group. In preeclampsia group, 20 women (10.3%) with gestational hypertension and mild preeclampsia were defined as “group 2”, and five women (2.5%) with severe preeclampsia and hemolysis, elevated liver enzymes, low platelet were defined as “group 3”. Maternal serum PAPP-A, PIGF, sEng levels were not significantly different between preeclampsia group and control group, whereas uterine artery Doppler pulsatility index (PI) values were significantly higher in preeclampsia group ($p=0.023$). sEng levels were significantly higher in group 3 than those in group 2 ($p=0.001$). If uterine artery PI cut-off level was taken as >2.23 in ROC curve analysis, sensitivity was 42.31% and specificity was 82.10% for detecting preeclampsia.

Conclusion: Maternal serum PAPP-A, PIGF and sEng were not effective in predicting preeclampsia. However, these markers can be used to distinguish between mild and severe preeclampsia. First trimester uterine artery Doppler examination is an effective screening method for predicting preeclampsia.

Keywords: Preeclampsia, uterine artery, pregnancy-associated plasma protein A, placental growth factor, sEndoglin

INTRODUCTION

Preeclampsia (PE) affects approximately 2% of all pregnancies and it is the most significant cause of maternal perinatal mortality and morbidity (1). Today, early detection of PE has become one of the fundamental goals of perinatal medicine. Although clinical symptoms of PE emerge after 20th week of gestation, trophoblast invasion that is responsible for pathogenesis occurs in the first trimester (2). High-resistant spiral arteries can be

detected from the 11th week of gestation by uterine artery (Uta) Doppler examination (3). Therefore, first trimester Uta Doppler examination may be a good noninvasive method for predicting PE that reflects abnormal trophoblast invasion. The addition of maternal history and biochemical serum markers to Uta Doppler examination is strongly associated with placental and endothelial dysfunction (4). These markers make screening relatively earlier, and most importantly, when used with first



Address for Correspondence: Şule Yıldırım Köpük, Ümraniye Training and Research Hospital, Clinic of Obstetrics and Gynecology, İstanbul, Turkey
Phone: +90 505 474 11 32 E-mail: suleyildirim@msn.com ORCID ID: orcid.org/0000-0002-5020-8323

Cite this article as: Yıldırım Köpük Ş, Çakıroğlu Y, Ceylan Y, Çekmen MB, Yücesoy G. Prediction of Preeclampsia by Uterine Artery Doppler Examination and Placental Growth Factor, Endoglin and Pregnancy-associated Plasma Protein Levels in Maternal Serum at 11-13+6 Pregnancy Week. Eur Arch Med Res 2019;35(3):137-42

©Copyright 2019 by the Health Sciences University, Okmeydanı Training and Research Hospital
European Archives of Medical Research published by Galenos Publishing House.

Received: 10.12.2017
Accepted: 03.09.2018

trimester Doppler sonography, they provide higher predictive value and more advanced diagnostic performance (5). Placental growth factor (PIGF) and vascular endothelial growth factor (VEGF) are potent angiogenic factors and they have a stimulating effect on endothelial cell proliferation and migration. Soluble fms-1like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) are anti-angiogenic factors and it is suggested that hypoxic placenta secretes increased amounts of sFlt-1 and sEng (6,7). Although there are contradictory results in the studies, it has been generally reported that the levels of angiogenic factors, PIGF and VEGF, are decreased in PE and the levels of anti-angiogenic factors, sFlt-1 and sEng, are increased (8). In addition, pregnancy-associated plasma protein A (PAPP-A) and free beta human chorionic gonadotropin (f-βhCG) have been used for chromosomal abnormality screening tests, and they have been also investigated in the prediction of complications that emerge in advanced gestational weeks in many studies and their availability has been discussed (9). Studies have shown that especially low PAPP-A levels may be closely associated with PE (10). Prediction of PE prediction has an important role in the prevention of complications associated with PE. Based on this reality, we intended to investigate the possible relationship between the development of PE development and maternal serum PIGF, sEng, PAPP-A, f-βhCG levels that were measured at 11+0 and 13+6 weeks of gestation and UtA pulsatility index (PI) values determined by Doppler ultrasonography.

METHODS

Singleton pregnant women who admitted for screening test at 11+0 and 13+6 weeks of gestation were included in the study. The study was planned as a prospective cohort study. Ethics committee approval was taken and cases were informed about the study. Exclusion criteria were determined as pregnancy greater than 14 weeks, multiple pregnancy, chromosomal or congenital fetal abnormality, chronic hypertension, molar pregnancy, type 1 diabetes mellitus, maternal renal disease, PE history and morbid obesity. Detailed maternal history was taken, ad nuchal translucency measurement and UtA Doppler examination were done. When consecutive similar wave patterns were obtained, PI was measured; mean PI values of right and left UtAs were calculated. Doppler measurement of the UtA PI at 11-13+6 weeks was performed by the same experienced sonographer. PAPP-A and f-βhCG were studied from blood samples. Measurements were performed using Immulite 2000 systems autoanalyzer with Siemens kits and by chemiluminescence method. The measurements were converted to multiple of median (MoM) values. Centrifuged blood samples

were stored at -80 °C to analyze PIGF and sEng levels. After the completion of the study groups, human sEng/CD105 ELISA (Aviscera Biscience, Inc. Santa Clara, USA) and PIGF ELISA (DRG Instruments GmbH, Germany) kits were tested using micro-ELISA method by Radim Aliysei instrument in stored serum samples.

Characteristics, medical history, pregnancy outcomes, sonography findings, systolic and diastolic blood pressure values, pregnancy complications, PIGF, sEng, MoM values of PAPP-A and f-βhCG of the cases were recorded in computer database. PE diagnosis was made when two different blood pressure measurements, measured every 4 hours, were 140/90 mmHg and above, and presence of 300 mg or more protein in the urine within 24 hours or presence of 30 mg/dL (1+dipstick) protein in spot urine sample following 20th weeks of pregnancy. According to ACOG criteria, PE group was divided into two groups as severe (group 3) and mild PE (group 2) (11). In case of only high blood pressure, gestational hypertension (GHT) was diagnosed; and in case of additional hemolysis, increase in liver enzymes and thrombocytopenia to PE-eclampsia table, hemolysis, elevated liver enzymes, low platelet (HELLP) was diagnosed (12). Group 1 was composed of pregnant women who did not develop complications and group 2 was composed of patients with the diagnosis of PE, GHT and HELLP.

Statistical Analysis

SPSS (Statistical Package of Social Sciences) 16 evaluation version was used in the analysis of collected data. Mann-Whitney U test was used for statistical assessment and chi-square test was used for the comparison of classifier data. The differences between mild PE, severe PE and control group in terms of independent variables were analyzed by Kruskal Wallis test. The correlations between the variables were determined by Spearman correlation analysis. Results were evaluated at 95% confidence interval and significance was evaluated at $p < 0.05$ level.

RESULTS

The study was performed on 250 pregnant women between 16-41 years of age who admitted to Kocaeli University Faculty of Medicine, Department of Obstetrics and Gynecology between February 2012 and 2014. Fifty-seven cases from whom we could not get pregnancy outcomes were excluded from the study. Out of patient group including 193 cases with serum PAPP-A, f-βhCG, PIGF and sEng measurements performed in 11-14 weeks of gestation and with known pregnancy outcomes, 168 women were not affected by PE, whereas PE was detected in 25 women including severe PE in 4 cases, mild PE in 18 cases, GHT in 2 cases and HELLP in one case. Patients with uncomplicated pregnancy

were classified as group 1 (n=168), and patients diagnosed with mild PE, severe PE, GHT and HELLP were classified as group 2 (n=25). None of the pregnant women included in the study had a history of smoking or pregnancy with assisted reproductive techniques in vitro fertilization. Demographic characteristics and pregnancy outcomes of the patients are given in Table 1. When preeclamptic and normotensive patients were compared based on maternal age, gravida, parity, body mass index, delivery week and values, no statistically significant difference was found ($p>0.05$) (Table 1). There was a significant difference in terms of birth weight of the newborn, mean arterial systolic pressure

Table 1. Demographic characteristics, serum biochemistry and ultrasonography results of the patients

Characteristics	Group 1 (control) (n=168)	Group 2 (preeclamptic) (n=25)	p
Maternal age (years)	28.5±5.90	30.03±6.39	0.494
Gravida	1.97±1.16	2.10±1.03	0.553
Parity	1.2±0.9	1.5±1.4	0.600
Body mass index (kg/m ²)	28.3±3.3	29.3±6.7	0.59
Delivery week	38.06±2.88	38.40±1.81	0.537
Weight of newborn (gr)	3080±53.51	2615±189.87	0.03
Mean systolic blood pressure	111.15±18.14	148.21±19.53	<0.0001
Mean diastolic blood pressure	70.54±9.45	98.05±13.18	<0.0001
PIGF (pg/mL)	71.25±0.9	67.24±1.1	0.571
sEndoglin (ng/mL)	15.38±0.2	15.82±0.3	0.642
PAPP-A (MoM)	1.2	1.01	0.417
f-βhCG (MoM)	1.2	0.91	0.184
UtA-PI	1.67±0.35	1.93±0.23	0.023
UtA-S/D	3.76±0.2	4.85±0.1	0.019

Values are mean ± standard deviation or n (%)

PIGF: Placental growth factor, PAPP-A: Pregnancy associated plasma protein-A, f-βhCG: Free chorionic gonadotropin, UtA-PI: Uterine artery pulsatility index measurement, UtA-S/D: Uterine artery systolic diastolic ratio, MoM: Multiple of median

and mean diastolic pressure ($p<0.05$). There was no statistically significant difference between preeclamptic and control groups in terms of PIGF, sEng, PAPP-A, f-βhCG serum markers. UtA PI ($p=0.023$) and systolic diastolic ($p=0.019$) ratio were significantly higher in the preeclamptic group. The groups are shown in Table 1. When we classified cases as control group (n=168), patients who developed mild PE and GHT (n=20) (group 2), and patients who developed severe PE and HELLP (n=5) (group 3), sEng level in group 3 was significantly higher than in group 2 (Table 2). PIGF level was relatively decreased in group 3 compared to group 2, however it was not statistically significant. PAPP-A MoM value was found to be significantly lower in group 3 compared to group 2. These results are summarized in Table 2. Prediction of PE could be made by ROC analysis with a PI cut-off value of >2.23, with a sensitivity of 42.31% and a specificity of 82.10%. It is summarized in Table 3 and Figure 1.

DISCUSSION

Prediction of PE in the first trimester, where maximum trophoblastic invasion occurs before the onset of the disease, is definitely an important goal. Therefore, many biochemical markers that can provide an estimate of PE are still being investigated. An ideal test should be simple, rapid, cost effective and easy to use. Positive likelihood ratio should be >15 and negative likelihood ratio should be <0.1. Its sensitivity and specificity should also be high. Today, there are no tests that are sufficient for PE prediction alone or in combination. While screening test which uses maternal obstetric history is quite

Table 3. Uterine artery pulsatility index cut-off value in preeclampsia prediction

	Cut-off PI	AUC %95 (CI)	LR+/LR-	Specificity	Sensitivity
Preeclampsia	>2.23	0.639 (0.566-0.708)	2.36/0.7	82.10%	42.31%

PI: Pulsatility index, AUC: Area under curve, CI: Confidence interval, LR: Likelihood ratio

Table 2. Comparison of serum markers and uterine artery pulsatility indexes between mild preeclampsia, severe preeclampsia and control groups

Median (25-75 p)	Group 2 (n=168)	Group 2 (n=20)	Group 3 (n=5)	p
PIGF (pg/mL)	71.25 (48.08-134.58)	76.05 (41.41-134.9)	66.1 (34.39-76.68)	0.24
sEndoglin (pg/mL)	1538 (1319-1732)	1497 (1232.75-1671.75)	1915 (1804-2212.5)	0.001
PAPP-A MoM	1.2 (0.81-1.72)	1.18 (0.71-1.93)	0.55 (0.45-0.86)	0.029
f-βhCG MoM	1.02 (0.69-1.51)	0.93 (0.55-1.33)	0.74 (0.52-1)	0.15
UtA-PI	1.67 (1.34-2.07)	1.84 (1.5-2.55)	2.07 (1.86-2.71)	0.023

PIGF: Placental growth factor, PAPP-A: Pregnancy associated plasma protein-A, f-βhCG: Free chorionic gonadotropin, UtA-PI: Uterine artery pulsatility index measurement, MoM: Multiple of median

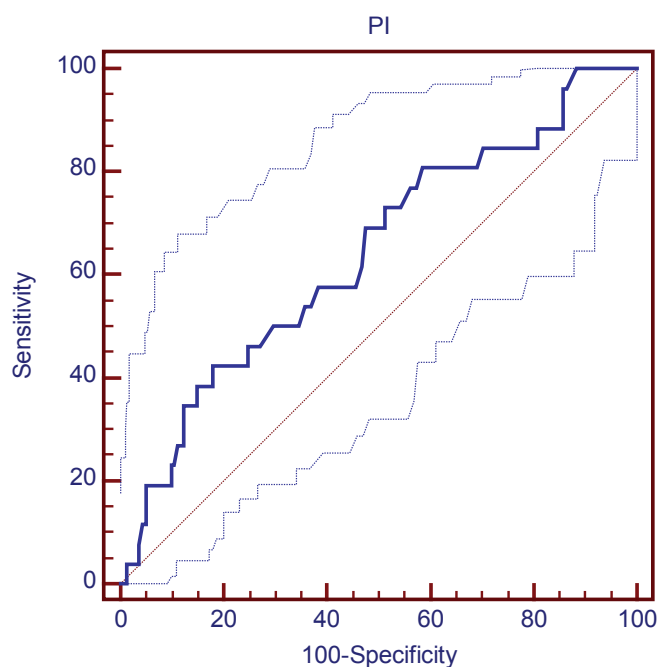


Figure 1. ROC curve analysis of uterine artery Doppler pulsatility index for prediction of preeclampsia

PI: Pulsatility index

insufficient, Harrington et al. (13) proved that PE screening might be possible by bilateral UtA Doppler examination with a high sensitivity and low specificity in the mid-1900s. In a large prospective study, the relationship between UtA Doppler findings [resistance index (RI) and diastolic notch] and the development of term and preterm PE at 11 and 14 weeks of gestation was investigated, and 57 term and 33 early PE cases were detected among 3058 pregnant women. The sensitivity of UtA Doppler examination in the detection of PE was found to be 49% within 90 percentile. In addition, it was indicated that when RI of UtA Doppler was greater than 90 percentile, there was a 6-fold increase in the likelihood of early PE. The mean RI values of early preeclamptic women were higher compared to preeclamptic women at term (14). In a retrospective study, first and second trimester UtA Doppler indices of 3560 pregnant women were investigated within a 7-year period and PE was detected in 126 pregnant women. It was determined by ROC analysis that PI value was the best predictor of PE. The difference between mean second trimester and first trimester PI values was found to be 0.851 in PE prediction before 34 gestational weeks and 0.786 for late PE, and the authors stated that the difference between two trimester mean PI values could be used in the prediction of PE (15). In our study, when UtA PI cut-off value was taken as >2.23 in the prediction of PE cases, sensitivity was 42.31% and specificity was 82.10%. In recent years, new screening protocols have been introduced to the literature by Fetal Medicine Foundation

with the combined evaluation of maternal characteristics, UtA Doppler and angiogenic-antiangiogenic markers (16). The addition of maternal history and biochemical serum markers to UtA Doppler examination was strongly associated with placental and endothelial dysfunction (4,5). Serum PIGF, sEng, sFlt-1 and placental protein 13 (PP-13) make it possible to make screening earlier, and most importantly, when used with first trimester Doppler sonography, they provide a higher predictive value and more advanced diagnostic performance (5). While PAPP-A alone provides a prediction of 10-20% during PE screening, it can provide a prediction of 70% [5% false positivity rate (FPR)] when combined with UtA Doppler indices (10,17). Spencer et al. (18) performed PAPP-A and PP-13 measurements in the first trimester and UtA Doppler examinations at 22-24 weeks of gestation. PAPP-A MoM value was found to be significantly lower in preeclamptic group compared to the control group. When PAPP-A was combined with UtA PI value, PE could be estimated with 76% sensitivity; however, there was no change in sensitivity with the addition of PP-13 to this combination. In their study, Di Lorenzo et al. (19) detected a significant relationship between early-onset PE and f- β hCG value above 3.0 MoM. However, many studies retrospectively evaluating f- β hCG have shown that it is unable to predict PE (10,20). In a study evaluating sEng level at 11-13 and 30-33 weeks of gestation, the median MoM value at 30-33 weeks of gestation was found to be higher in preeclamptic group compared to control group, and sEng level at 11-13 weeks of gestation was found to be similar between the two groups. When maternal characteristics were combined with third trimester sEng levels, late-onset PE prediction was reported as 50% with a FPR of 10% (21). PIGF is a proangiogenic protein and PE has been associated with the production of smaller amounts of this protein. Decrease in concentration may occur during clinical symptoms and preclinical period of the disease, and it is present since first trimester. In the first trimester, preeclamptic pregnant women were found to have low PIGF concentration and it was inversely proportional to the severity of the disease (22). No difference was found between normotensive and preeclamptic patients in terms of PIGF levels tested in urine at 8-21 weeks of pregnancy. Regarding PE prediction, preterm PIGF level in the 21-32 weeks of pregnancy was higher compared to term PIGF level (23). Akolekar et al. (24) examined UtA PI, mean blood pressure, PIGF, PAPP-A values in 58.884 cases at 11-13 weeks of pregnancy. They reported a PE detection rate of 50% with a FPR of 10% by using only maternal body mass index (BMI) and mean artery pressure at 34 weeks of pregnancy and a detection rate of 75% with the addition of UtA PI and more than 95% with the addition of PIGF and PAPP-A values (24). However, Diguisto et

al. (25) reported that the multivariate model adjusted according to laboratory and sonographic indicators had an area under the curve (AUC) estimated at 0.76, which was not significantly different from the AUC of the univariate model adjusted only for PIGF ($p=0.7$). As a result, in a high-risk population, PIGF in the first trimester is useful for predicting PE, but neither sFlt-1 nor any UtA Doppler indices improved the prediction of PE (25). Li et al. (26) demonstrated that UtA PI at early second-trimester was increased in pregnancies that developed PE (1.61 ± 0.047 vs. 1.02 ± 0.049 , $p<0.001$). In contrast, the level of PIGF was decreased in preeclamptic group compared to controls (0.69 ± 0.23 vs. 1.00 ± 0.26 , $p<0.001$) (26).

UtA PI, PAPP-A, PIGF, PP-13, inhibin-A, activin-A, sEng and mean arterial pressure were studied in 33, 602 pregnant women at 11-13 weeks of pregnancy. When only maternal factors (history), UtA PI, blood pressure and PAPP-A were evaluated in PE cases, early PE was estimated by 33% with a FPR of 5%, PE between 34-37 weeks was estimated by 27.8% and PE after 37 weeks was estimated by 24.5%. PE prediction rate was reported as 91%, 79.4% and 60.9%, respectively, with the addition of other biochemical markers to maternal factors (4). In combined screening with maternal factors, mean arterial pressure, UtA PI and PIGF detection rate was 100% [95% confidence interval (CI) 80-100] for PE at <32 weeks, 75% (95% CI 62-85) for PE at <37 weeks and 43% (95% CI 35-50) for PE at ≥ 37 weeks, at 10% FPR. The combination of maternal factors and biomarkers provides effective first trimester screening for preterm PE (27). Poon et al. (28) investigated 7797 singleton pregnant women at 11-13 weeks of pregnancy. The authors predicted that early-onset PE would develop in 476 patients by logistic regression analysis using variables of UtA PI, mean blood pressure, serum PAPP-A, PIGF, BMI, nulliparity and a previous history of PE in previous pregnancy; however, PE developed in 32 patients. While 7321 patients were expected not to develop PE, two patients had a diagnosis of PE. As a result, the sensitivity and specificity of this model were calculated as 94.1% and 94.3%, respectively (28). In our study, when we classified the patients as group 1 (control group, $n=168$), group 2 (patients with mild PE and GHT; $n=20$) and group 3 (patients with severe PE and HELLP; $n=5$), median sEng value was 14.97 ng/mL in group 2 and 19.15 ng/mL in group 3, and a statistically significant difference was detected between the groups ($p=0.001$). sEng level in group 1 was higher than in group 2. PIGF level was relatively decreased and was 76.05 pg/mL in group 2 and 66.10 pg/mL in group 3, however, there was no statistically significant difference. Similar to the literature, sEng was statistically higher and PIGF was lower between group 2 and 3. Unlike the literature, low PIGF and high sEng

levels were detected in the control group. The disproportionate distribution of cases between groups (168/5, 168/20) and fewer preeclamptic patients may be the reason of this situation. While PIGF and PAPP-A levels decrease in PE, sEng level increases. In our study, no statistically significant difference was found between the preeclamptic group and control group in terms of these markers. We consider that this result is due to the fact that the distribution of cases was disproportionate and the number of preeclamptic cases was less than the control group. However, in subgroup analysis, it was found that PIGF level in the group 3 was significantly decreased and sEng levels were increased compared to group 2. UtA PI value was higher in preeclamptic pregnant women compared to the control group. Similar results were obtained in our study. PI value in preeclamptic group was significantly higher compared to control group ($p=0.023$). When PI cut-off value was taken as >2.23 in PE prediction, the sensitivity and specificity were 42.31% and 82.10%, respectively. UtA Doppler examination is an effective diagnostic tool in PE prediction.

CONCLUSION

Since UtA Doppler screening is simple and noninvasive and brings very little extra time to the duration of screening, it may be included in the current sonographic examination. The sensitivity of UtA Doppler examination may be increased by adding one or more serum markers. Combinations provide an increase in the ability of prediction and determination compared to the tests used alone.

Ethics

Ethics Committee Approval: The study was approved by the KAEK (project no: 2013/100, date: 26.03.2013).

Informed Consent: Informed consent of all patients were taken.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ş.Y.K., Concept: Ş.Y.K., G.Y., Design: Y.Ç., M.B.Ç., G.Y., Data Collection or Processing: Ş.Y.K., Y.C., Analysis or Interpretation: Y.Ç., Ş.Y.K., Literature Search: Ş.Y.K., Y.C., G.Y., Writing: Ş.Y.K., G.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. ACOG Committee on Obstetric Practice. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists. *Int J Gynaecol Obstet* 2002;77:67-75.
2. Caniggia I, Winter J, Lye SJ, Post M. Oxygen and placental development during the first trimester: implications for the pathophysiology of pre-eclampsia. *Placenta* 2000;21 Suppl A:25-30.
3. Gómez O, Figueras F, Martínez JM, del Río M, Palacio M, Eixarch E, et al. Sequential changes in uterine artery blood flow pattern between the first and second trimesters of gestation in relation to pregnancy outcome. *Ultrasound Obstet Gynecol* 2006;28:802-8.
4. Akolekar R, Syngelaki A, Sarquis R, Zvanca M, Nicolaides KH. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks. *Prenat Diagn* 2011;31:66-74.
5. Baumann MU, Bersinger NA, Surbek DV. Serum markers for predicting pre-eclampsia. *Mol Aspects Med* 2007;28:227-44.
6. Chaiworapongsa T, Romero R, Tarca AL, Kusanovic JP, Gotsch F, Mittal P, et al. A decrease in maternal plasma concentrations of sVEGFR-2 precedes the clinical diagnosis of preeclampsia. *Am J Obstet Gynecol* 2010;202:550.e1-10.
7. Luft FC. Soluble endoglin (sEng) joins the soluble fms-like tyrosine kinase (sFlt) receptor as a preeclampsia molecule. *Nephrol Dial Transplant* 2006;21:3052-4.
8. Al-Jameil N, Aziz Khan F, Fareed Khan M, Tabassum H. A Brief Overview of Preeclampsia. *J Clin Med Res* 2014;6:1-7.
9. Krantz D, Goetzl L, Simpson JL, Thom E, Zachary J, Hallahan TW, et al. Association of extreme first trimester free human chorionic gonadotropin-beta, pregnancy associated plasma protein A, and nuchal translucency with intrauterine growth restriction and other adverse pregnancy outcomes. *Am J Obstet Gynecol* 2004;191:1452-8.
10. Goetzing KR, Singla A, Gerkowicz S, Dicke JM, Gray DL, Odibo AO. Predicting the risk of pre-eclampsia between 11 and 13 weeks' gestation by combining maternal characteristics and serum analytes, PAPP-A and free β -hCG. *Prenat Diagn* 2010;30:1138-42.
11. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol* 2013;122:1122-31.
12. Sibai BM, Ramadan MK, Chari RS, Friedman SA. Pregnancies complicated by HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets): subsequent pregnancy outcome and long-term prognosis. *Am J Obstet Gynecol* 1995;172:125-9.
13. Harrington K, Carpenter RG, Goldfrad C, Campbell S. Transvaginal Doppler ultrasound of the uteroplacental circulation in the early prediction of pre-eclampsia and intrauterine growth retardation. *Br J Obstet Gynaecol* 1997;104:674-81.
14. Melchiorre K, Wormald B, Leslie K, Bhide A, Thilaganathan B. First-trimester uterine artery Doppler indices in term and preterm pre-eclampsia. *Ultrasound Obstet Gynecol* 2008;32:133-7.
15. Napolitano R, Melchiorre K, Arcangeli T, Dias T, Bhide A, Thilaganathan B. Screening for preeclampsia by using changes in uterine artery Doppler indices with advancing gestation. *Prenat Diagn* 2012;32:180-4.
16. 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.
17. 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.
18. Spencer K, Cowans NJ, Chefetz I, Tal J, Meiri H. First-trimester maternal serum PP-13, PAPP-A and second-trimester uterine artery Doppler pulsatility index as markers of pre-eclampsia. *Ultrasound Obstet Gynecol* 2007;29:128-34.
19. Di Lorenzo G, Ceccarello M, Cecotti V, Ronfani L, Monasta L, Vecchi Brumatti L, et al. First trimester maternal serum PIGF, free β -hCG, PAPP-A, PP-13, uterine artery Doppler and maternal history for the prediction of preeclampsia. *Placenta* 2012;33:495-501.
20. Spencer K, Yu CK, Cowans NJ, Otiqbah 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;25:949-53.
21. Lai J, Syngelaki A, Poon LC, Nucci M, Nicolaides KH. Maternal serum soluble endoglin at 30-33 weeks in the prediction of preeclampsia. *Fetal Diagn Ther* 2013;33:149-55.
22. Kusanovic JP, Romero R, Chaiworapongsa T, Erez O, Mittal P, Vaisbuch E, et al. 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;22:1021-38.
23. Levine RJ, Thadhani R, Qian C, Lam C, Lim KH, Yu KF, et al. Urinary placental growth factor and risk of preeclampsia. *JAMA* 2005;293:77-85.
24. Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing risks model in early screening for preeclampsia by biophysical and biochemical markers. *Fetal Diagn Ther* 2013;33:8-15.
25. Diguisto C, Piver E, Gouge AL, Eboue F, Vaillant CL, Maréchaud M, et al. First trimester uterine artery Doppler, sFlt-1 and PIGF to predict preeclampsia in a high-risk population. *J Matern Fetal Neonatal Med* 2017;30:1514-9.
26. Li L, Zheng Y, Zhu Y, Li J. Serum biomarkers combined with uterine artery Doppler in prediction of preeclampsia. *Exp Ther Med* 2016;12:2515-20.
27. O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, Wright A, et al. Accuracy of competing-risks model in screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. *Ultrasound Obstet Gynecol* 2017;49:751-755.
28. Poon LC, Kametas NA, Maiz N, Akolekar R, Nicolaides KH. First-trimester prediction of hypertensive disorders in pregnancy. *Hypertension* 2009;53:812-8.