Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/medici

Original Research Article

sFlt-1, PlGF, sFlt-1/PlGF ratio and uterine artery Doppler for preeclampsia diagnostics

Viktorija Tarasevičienė [a,*], *Regina Grybauskienė* [b], *Regina Mačiulevičienė* [a]

[a] *Department of Obstetrics and Gynecology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania*
[b] *Institute of Cardiology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania*

ARTICLE INFO

Article history:
Received 2 July 2015
Accepted 18 November 2016
Available online 29 November 2016

Keywords:
Preeclampsia
sFlt-1
PlGF
sFlt-1/PlGF
Uterine artery Doppler

ABSTRACT

Background and objective: Angiogenic factors such as soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) play a key role in the pathogenesis of preeclampsia. Uterine artery (UA) blood flow is important for preeclamptic pregnancy outcome, but small amount of evidence suggests UA dopplerometry for preeclampsia diagnostics and management. The aim of our study was to compare the value of angiogenic factors and UA dopplerometry in preeclampsia diagnosis and determine cut-off values to obtain the highest sensitivity and specificity of the parameter.

Materials and methods: We performed a case controlled study of 72 pregnant women with preeclampsia and 72 healthy matched controls. SFlt-1 and PlGF were measured in serum samples, the sFlt-1/PlGF ratio was calculated and UA pulsatility (PI) and resistance (RI) indexes were registered.

Results: Significantly higher levels of sFlt-1, sFlt-1/PlGF ratio and mean UAPI and UARI and lower levels of PlGF were found in preeclampsia group when compared to controls. The highest sensitivity and specificity for preeclampsia had SFlt-1/PlGF and PlGF with the cut-off values of ≥35 (sensitivity of 95.8% and specificity of 96.2%, respectively) and ≤138.6 pg/mL (sensitivity of 95.8% and specificity of 93.7%, respectively). For diagnostics of early-onset preeclampsia, all factors sFlt-1, PlGF and sFlt-1/PlGF had equal significance with the cut-off values of ≥7572 pg/mL (specificity of 97.5%, sensitivity 92.3%), ≤100.5 pg/mL (specificity 96.2%, sensitivity of 100%) and ≥54.6 (specificity 97.5%, sensitivity 97.5%) respectively.

Conclusions: The sFlt-1/PlGF ratio and PlGF are superior to sFlt-1, UAPI and UARI for preeclampsia diagnosis. For early-onset preeclampsia diagnostics either sFlt-1 or PlGF is sufficient.

* *Corresponding author at*: K. Genio 17-1, 50167 Kaunas, Lithuania.
E-mail address: viktorija.taraseviciene@gmail.com (V. Tarasevičienė).
Peer review under the responsibility of the Lithuanian University of Health Sciences.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.medici.2016.11.008

1. Introduction

Preeclampsia is pregnancy-induced disease that is associated with maternal and perinatal morbidity and mortality. According to the literature reports, it occurs in 2%–8% of all pregnancies [1]. In clinical practice, preeclampsia is diagnosed when after the 20th gestational week, hypertension (systolic blood pressure 140 mmHg or more and diastolic blood pressure 90 mmHg or more, when measured twice in a period of not less than 6 h) and proteinuria (more than 300 mg/L in a 24-h period) commence. In more severe cases headache, impaired vision, epigastric pain, thrombocytopenia, hemolysis or abnormal liver and renal function can occur. But sometimes preeclampsia can present with atypical manifestation. For example, HELLP syndrome, which occurs in severe preeclampsia, can present without proteinuria, and on the contrary, proteinuria can be associated with diabetes mellitus, kidney disease and systemic connective tissue diseases even if hypertension is present. Thus more precise methods for diagnosing preeclampsia would be of a great value.

It is known that before clinical onset of preeclampsia trophoblastic invasion is impaired and placental perfusion is disturbed [2,3]. The impaired placental blood flow can be confirmed by uterine artery blood flow Doppler examination.

Recent studies suggest that before clinical manifestation of preeclampsia, placenta releases soluble angiogenic factors, which play the crucial role in endothelial dysfunction and development of preeclampsia related symptoms [4]. One of those factors is soluble fms-like tyrosine kinase-1 (sFlt-1). It binds vascular endothelial growth factor A (VEGF-A) and placental growth factor (PlGF) which are the main angiogenic factors, responsible for placental vascular development and maternal endothelial function, and in this way prevents their interaction with endogenous receptors in the vessels [5]. Because of that circulating PlGF levels are decreased. sFlt-1 levels are elevated [6]. According to the literature, the ratio of these two factors is more accurate for diagnosing and prediction of preeclampsia than any of these factors alone [1,7–9]. However, the cut-off values of these angiogenic factors for diagnosing preeclampsia are still unclear. The accuracy and precision of angiogenic factor levels in the blood of preeclamptic women compared to Doppler flow of uterine arteries have not been established yet.

The aim of our study is to investigate and compare the value of angiogenic factors and uterine artery dopplerometry in diagnosing preeclampsia and determine cut-off values to reach highest sensitivity and specificity.

2. Materials and methods

A case-control clinical study was performed in the tertiary care center of the Hospital of Lithuanian University of Health Sciences Kauno Klinikos. We included 144 pregnant women at 26–40 weeks of gestation, who gave a written informed consent during the period of October 2010–June 2011. Approval by the local ethics committee was obtained.

The study group consisted of 72 women who were consecutively admitted to Kaunas Perinatal Centre because of preeclampsia diagnosis. Women who had other pregnancy disorders or adjacent diseases and who came after the rupture of the amniotic membranes or delivering were excluded from the study. A total of 72 healthy pregnant women matched for age, body mass index (BMI), and gestational age comprised the control group.

Preeclampsia was diagnosed on the basis of a new onset of hypertension and proteinuria after 20 weeks of gestation [10]. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg measured twice, not less than 6 h apart. Proteinuria was defined as excretion of ≥300 mg of protein in 24-h urine collection.

Early preeclampsia was classified when occurred before 34 weeks of gestation and late, at 34 weeks of gestation and later on [11].

Maternal blood in preeclampsia group was collected on the day the diagnosis was confirmed and in matched control group, during their visit to outpatient department by venipuncture in tubes without anticoagulant. After clotting, the samples were centrifuged and serum was pipetted and stored at −40 °C until testing. The sFlt-1 and PlGF concentrations of each sample were tested in parallel on the fully automated Roche Elecsys system. For each sample, the sFlt-1/PlGF ratio was calculated.

Uterine artery Doppler sonography was performed on the same day the blood samples were collected. Toshiba Applio ultrasound machine was used for all Doppler measurements. Uterine artery waveforms were obtained with an abdominal probe, 1 cm from the uterine artery optical crossover with external iliac artery on both sides. Uterine artery pulsatility (PI) and resistance (RI) indexes were measured on both sides and mean uterine artery PI and RI calculated. Clinicians were blinded for angiogenic test results.

Statistical analysis was performed with SPSS 17.0 for Windows. As the distribution of angiogenic factors and uterine artery resistance indexes was abnormal, we used non parametric criteria for describing and comparing the groups. We calculated median values with interquartile ranges of sFlt-1, PlGF, sFlt-1/PlGF ratio, mean uterine artery PI and RI in preeclampsia and control groups. For comparison of numerical variables between two independent groups, nonparametric Mann–Whitney criterion was used. Categorical variables were expressed using frequency measures; comparison between groups was made with the help of chi-square (χ^2) test. For determination of specificity and sensitivity of sFlt-1, PlGF, sFlt-1/PlGF ratio, uterine artery PI and RI receiver operating curve analysis was performed. Differences were considered to be significant when $P < 0.05$.

3. Results

The general characteristics of the included participants are demonstrated in Table 1. We found no differences in the maternal age, BMI and gestational age between women who had preeclampsia and controls. However, there were more primiparous women in preeclampsia group. Thus separate comparison analysis of angiogenic factor values between groups was performed for primiparous women only, but results did not differ significantly from the comparison of the whole group.

Table 1 – Characteristics of the study population.

Factor	Preeclampsia group (N = 72)	Control group (N = 72)	P
Age, years	29.6 (25.2–34)	30 (26–36.7)	0.06
BMI, kg/m^2	25 (21.8–32.2)	26.2 (22.6–32.8)	0.21
Gestational age, weeks	34 (30–38)	34 (30–38)	0.55
Primiparas, *n* (%)	44 (61.1)	29 (36.3)	0.003
Systolic blood pressure, mmHg[a]	160 (160–180)	130 (120–144.5)	<0.0001
Diastolic blood pressure, mmHg[a]	110 (100–110)	80 (70–90)	<0.0001

Values are presented as median (interquartile range) unless otherwise indicated.
[a] Systolic and diastolic blood pressure on investigation day.

Table 2 – sFlt-1 and PlGF levels, their ratio and uterine artery PI and RI in the preeclampsia and control groups.

Factor	Preeclampsia group (N = 72)	Control group (N = 72)	P
sFlt-1, pg/mL	9581 (6906–15,738)	1731.5 (981.9–3086.7)	<0.0001
PlG, pg/mL	62.6 (40.4–92.7)	346.4 (204.9–546.0)	<0.0001
sFlt-1/PlGF	158 (74.7–330)	3.96 (2.4–12.1)	<0.0001
Uterine artery PI	1.14 (0.9–1.4)	0.76 (0.6–0.9)	<0.0001
Uterine artery RI	0.58 (0.52–0.72)	0.49 (0.42–0.53)	<0.0001

Values are presented as median with interquartile range.

In preeclampsia group women had higher systolic and diastolic blood pressure and median proteinuria of 2 g (1.6–3.1).

Comparing sFlt-1, sFlt-1/PlGF ratio, mean uterine artery PI and RI between preeclampsia and control groups, significantly higher values were found in preeclampsia group (Table 2). PlGF values were significantly lower respectively.

Uterine artery end diastolic notches in the preeclampsia group were registered in 61% of the women ($n = 44$): unilateral in 28.8% ($n = 20$) and bilateral in 33.3% ($n = 24$). They were registered in 12.5% of the control women ($n = 10$): unilateral in 8.8% ($n = 7$) and bilateral in 3.7% ($n = 3$) ($P < 0.001$).

The results from receiver operating curve analysis determining the cut-offs with the highest sensitivity and specificity for preeclampsia are presented in Figs. 1 and 2. Areas under the curve for sFlt-1, PlGF, sFlt-1/PlGF, uterine artery PI and RI were 0.954, 0.977, 0.983, 0.779, and 0.764, respectively. ROC curve comparison analysis showed significantly greater specificity and sensitivity of sFlt-1/PlGF and PlGF over sFlt-1, mean uterine artery PI and RI for preeclampsia diagnostics. There was no significant difference in specificity and sensitivity between sFlt-1/PlGF and PlGF ($P = 0.8$). The cut-off values of sFlt-1, PlGF, sFlt-1/PlGF, uterine artery PI and RI for preeclampsia diagnostics are presented in Table 3.

Separate analysis for early-onset preeclampsia diagnosis showed that areas under the curve for sFlt-1, PlGF, sFlt-1/PlGF, uterine artery PI and RI were respectively 0.981, 0.998, 0.997,

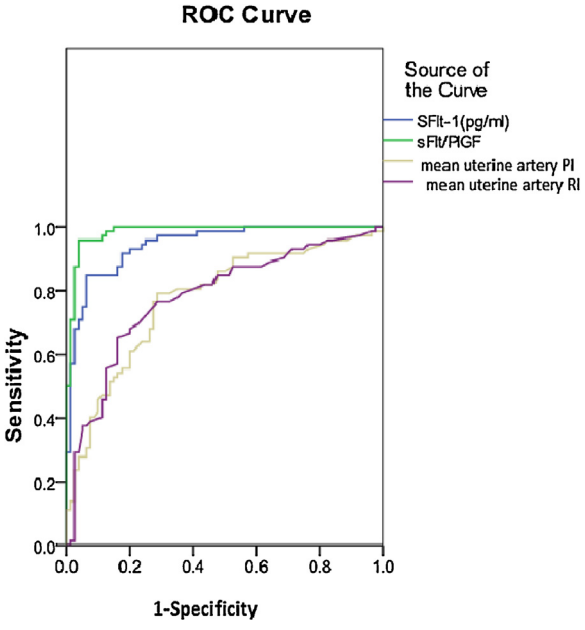

Fig. 1 – ROC curves of sFlt-1, sFlt-1/PlGF and mean uterine artery PI and RI for diagnosing preeclampsia.

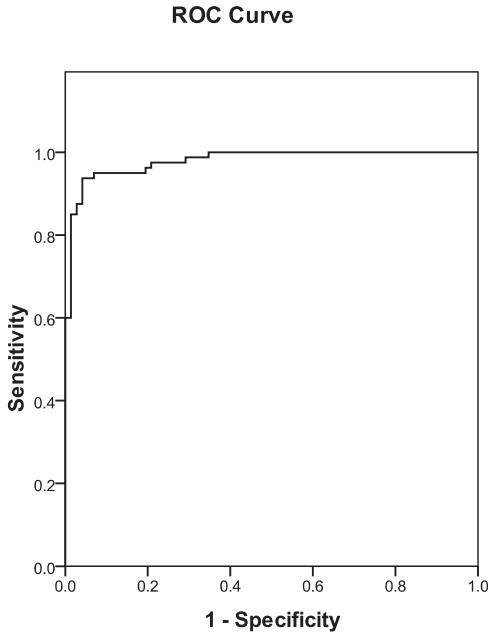

Fig. 2 – ROC curve of PlGF (pg/mL) for preeclampsia diagnostics.

Table 3 – Sensitivity and specificity of PlGF, sFlt-1, sFlt-1/PlGF and mean uterine artery PI and RI for diagnosing preeclampsia.

Factor	Sensitivity, %	Specificity, %
sFlt-1 > 6326 pg/mL	84.7	65.8
PlGF ≤ 138.6 pg/mL	95.8	93.7
sFlt-1/PlGF > 34.93	95.8	96.2
Mean uterine artery PI > 0.89	79.2	71.2
Mean uterine artery RI > 0.55	65.3	83.7

0.888, and 0.907 (Figs. 3 and 4). Comparison of ROC curves showed that there was no significant difference in sFlt-1, PlGF and sFlt-1/PlGF ratio areas under the ROC curves for early-onset preeclampsia diagnostics. The cut-off values for diagnosing early-onset preeclampsia are presented in Table 4.

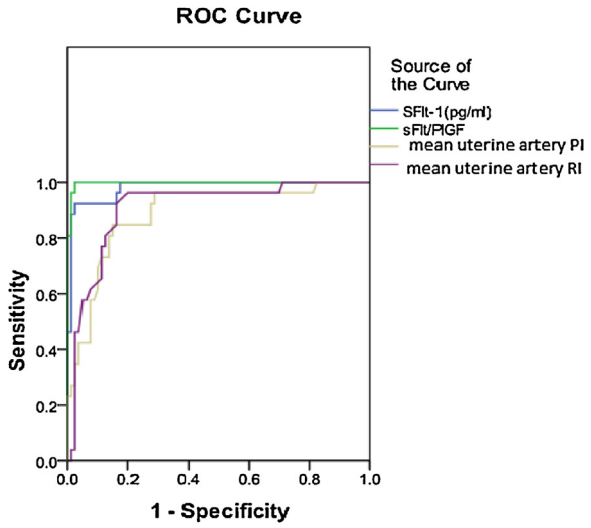

Fig. 3 – ROC curves of sFlt-1, sFlt-1/PlGF and mean uterine artery PI and RI for diagnosing early-onset preeclampsia.

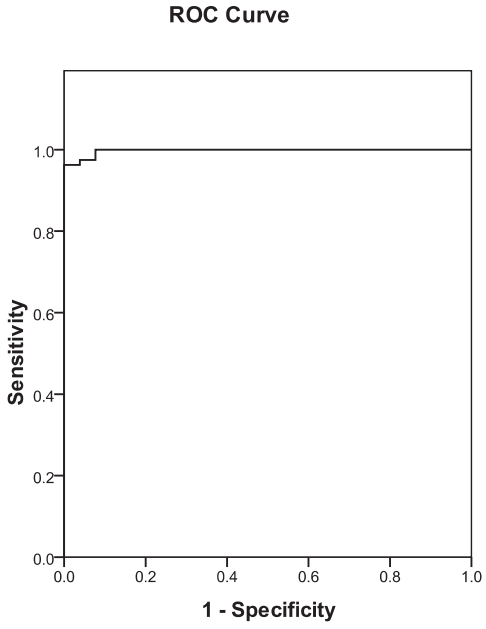

Fig. 4 – ROC curve of PlGF for early-onset preeclampsia diagnostics.

Table 4 – Sensitivity and specificity of PlGF, sFlt-1, sFlt-1/PlGF and mean uterine artery PI and RI for diagnosing early-onset preeclampsia.

Factor	Sensitivity, %	Specificity, %
sFlt-1 > 7572 pg/mL	92.3	97.5
PlGF ≤ 100.5 pg/mL	100	96.2
sFlt-1/PlGF > 54.63	97.5	97.5
Mean uterine artery PI > 1.095	85	84.6
Mean uterine artery RI > 0.54	96.2	80

4. Discussion

Clinical presentation of preeclampsia can differ enormously. A clinician's goal is to make a correct diagnosis in the shortest period of time. The aim of this study was to find, which method: uterine artery dopplerometry or angiogenic factor measurement is superior in diagnosing preeclampsia and which cut-off should be used.

Our study showed that ultrasound examination alone has lower value in diagnosing early-onset preeclampsia as well as any preeclampsia if compared to angiogenic factors. The findings are compatible with those, found in the literature [12,13]. According to Ghi et al., Meler et al. and Simanavičiūtė, uterine artery dopplerometry can be more helpful for prediction of adverse neonatal and maternal outcomes in patients with preeclampsia but not for determining diagnosis [14–16].

Similarly as in other scientist studies we found significantly higher sFlt-1 and lower PlGF levels in the blood of preeclamptic women as compared to healthy controls [7,17–19]. The SFlt-1/PlGF ratio had the highest sensitivity and specificity (95.8% and 96.2%) for diagnosing preeclampsia with a cut-off value of 35. It is known that an increase in the sFlt-1/PlGF ratio is associated with the severity of the disease and the values differ in cases of early and late preeclampsia. But our goal was to find a cut-off point that is irrespective of gestational age and severity of the disease. The one above which preeclampsia diagnosis should be considered and confirmed or ruled out by other tests.

The limitation of our study is a quite small group of preeclamptic patients. Some authors also presented similar results regarding the sensitivity and specificity of sFlt-1/PlGF ratio [7–9], but their reported cut-off values were different from our findings. Verlohren et al. and Gomez-Arriaga et al. reported that the sFlt-1/PlGF ratio of 85 sufficiently discriminated women who have preeclampsia from healthy controls [7,12]. Rolfo et al. compared the sFlt-1/PlGF ratio in women who had preeclampsia and women who had renal diseases with proteinuria and concluded that preeclampsia can be diagnosed when the sFlt-1/PlGF ratio is above 148.75 [8]. The difference of the cut-offs can also be explained by different severity of the disease in the included population. Moreover, we did not have intrauterine growth restricted fetuses without preeclampsia in our control group–in such cases angiogenic factors differ from factors in normal pregnancy and the cut-off value for preeclampsia could be found higher [12].

Our results showed that PlGF alone with the cut-off value of less than 138 pg/mL had a specificity of 93.7% and a sensitivity of 95.8% for diagnosing preeclampsia. No significant difference between ROC curves of sFlt-1/PlGF and PlGF were found. Thus, according to our results, PlGF alone might have similar value

for diagnosing preeclampsia. This is of importance in terms of reducing costs.

For early preeclampsia diagnostics all angiogenic factors were recognized equally significant with greater sensitivity and specificity as compared to the whole group. The cut-off values of sFlt-1/PlGF >54.6 pg/mL, sFlt-1 >7572 pg/mL and PlGF of <100.5 pg/mL were found to diagnose early-onset preeclampsia. According to our material in early-onset preeclampsia the evaluation of one of angiogenic factors is sufficient to diagnose the disease. This is in concordance with Gómez-Arriaga et al., who reported that testing either sFlt-1 or PlGF for early preeclampsia diagnostics is enough [12]. This can be explained by the fact that in the pathogenesis of early-onset preeclampsia defective placentation, abundant secretion of soluble angiogenic factors and endothelial dysfunction is crucial; however, in late-onset preeclampsia the pathogenetic mechanism differs.

According to our results implementation of PlGF or sFlt-1/PlGF ratio investigation into clinical practice for preeclampsia diagnosis is most valuable, when decision of admitting to the hospital and transportation of premature fetus to perinatal center has to be made. Especially great importance of angiogenic factors could be in cases of atypical preeclampsia. But still further studies with bigger sample size are necessary.

5. Conclusions

We conclude that the sFlt-1/PlGF ratio and PlGF alone are superior to uterine artery PI and RI for establishing preeclampsia diagnosis. The diagnosis of preeclampsia must be ruled out, when the cut-off value of sFlt-1/PlGF ratio is ≥35 or PlGF value is equal to or less than 138 pg/mL. In cases of early-onset preeclampsia, the cut-off values of sFlt-1 was >7572 pg/mL; PlGF, <100.5 pg/mL; and sFlt-1/PlGF ratio, >54.6 pg/mL. The analysis of only one angiogenic factor was sufficient to estimate preeclampsia diagnosis on its early onset.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

[1] Kim SY, Ryu HM, Yang JH, Kim MY, Han JY, Kim JO, et al. Increased sFlt-1 to PlGF ratio in women who subsequently develop preeclampsia. J Korean Med Sci 2007;22(5):873–7.

[2] Lin S, Shimizu I, Suehara N, Nakayama M, Aono T. Uterine artery Doppler velocimetry in relation to trophoblast migration into the myometrium of the placental bed. Obstet Gynecol 1995;85:760–5.

[3] Kaar K, Jouppila P, Kuikka J, Luotola H, Toivanen J, Rekonen A. Intervillous blood flow in normal and complicated late pregnancy measured by means of an intravenous 133Xe method. Acta Obstet Gynecol Scand 1980;59:7–10.

[4] Fisher SJ. The placental problem: linking abnormal cytotrophoblast differentiation to the maternal symptoms of preeclampsia. Reprod Biol Endocrinol 2004;2:53.

[5] Venkatesha S, Toporsian M, Lam C. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med 2006;12:642–9.

[6] Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med 2006;355(10):992–1005.

[7] Verlohren S, Galindo A, Schlembach D, Zeisler H, Herraiz I, Moertl MG, et al. An automated method for the determination of the sFlt-1/PIGF ratio in the assessment of preeclampsia. Am J Obstet Gynecol 2010;202(2):161.e1–161.e11.

[8] Rolfo A, Attini R, Nuzzo AM, Piazzese A, Parisi S, Ferraresi M, et al. Chronic kidney disease may be differentially diagnosed from preeclampsia by serum biomarkers. Kidney Int 2013;83(1):177–81.

[9] Sunderji S, Gaziano E, Wothe D, Rogers LC, Sibai B, Karumanchi SA, et al. Automated assays for sVEGF R1 and PlGF as an aid in the diagnosis of preterm preeclampsia: a prospective clinical study. Am J Obstet Gynecol 2010;202(1):40.e1–7.

[10] 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.

[11] Valensise H, Vasapollo B, Gagliardi G, Novelli GP. Early and late preeclampsia: two different maternal hemodynamic states in the latent phase of the disease. Hypertension 2008;52(5):873–80.

[12] Gómez-Arriaga PI, Herraiz I, López-Jiménez EA, Gómez-Montes E, Denk B, Galindo A. Uterine artery Doppler and sFlt-1/PlGF ratio: usefulness in diagnosis of pre-eclampsia. Ultrasound Obstet Gynecol 2013;41(5):530–7.

[13] Crispi F, Domínguez C, Llurba E, Martín-Gallán P, Cabero L, Gratacós E. Placental angiogenic growth factors and uterine artery Doppler findings for characterization of different subsets in preeclampsia and in isolated intrauterine growth restriction. Am J Obstet Gynecol 2006;195(1):201–7.

[14] Ghi T, Youssef A, Piva M, Contro E, Segata M, Guasina F, et al. The prognostic role of uterine artery Doppler studies in patients with late-onset preeclampsia. Am J Obstet Gynecol 2009;201(1):36.

[15] Meler E, Figueras F, Mula R, Crispi F, Benassar M, Gómez O, et al. Prognostic role of uterine artery Doppler in patients with preeclampsia. Fetal Diagn Ther 2010;27(1):8–13.

[16] Simanavičiūtė D. Sergančiųjų hipertenzija gimdos ir vaisiaus kraujotakos pokyčiai [The changes of uterine and fetal blood flow in pregnant women with hypertensive disorders].[dissertation]. Kaunas: KMU; 2003.

[17] Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu K, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672–83.

[18] Schiettecatte J, Russcher H, Anckaert E, Mees M, Leeser B, Tirelli AS, et al. Multicenter evaluation of the first automated Elecsys sFlt-1 and PlGF assays in normal pregnancies and preeclampsia. Clin Biochem 2010;43:768–70.

[19] Woolcock J, Hennessy A, Xu B, Thornton C, Tooher J, Makris A, et al. Soluble Flt-1 as a diagnostic marker of pre-eclampsia. Aust N Z J Obstet Gynaecol 2008;48(1):64–70.