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DIGITALNI AKADEMSKI ARHIVI I REPOZITORIJI

ORIGINAL ARTICLE

The soluble fms-like tyrosin kinase-1 (sFLT-1) to placental growth factor (PlGF) ratio as a possible indicator for the severity of preeclampsia - single institution experience

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ABSTRACT

Aim To investigate a potential of the clinical use of the soluble fms-like tyrosine kinase 1 (sFLT-1) to placental growth factor (PlGF) ratio from the perspective of a small hospital centre.

Methods Maternal serum samples were analysed at $24^{1/7}$-$28^{0/7}$, and $28^{1/7}$-$32^{0/7}$ weeks of gestation**.** The level of sFLT-1 and PlGF was determined by immunoassay platform and used to calculate the sFLT-1/PlGF ratio in 35 pregnant women, and divided into subgroups according to preeclampsia occurrence at the time of delivery: preterm (≤37 weeks) or term (37-42 weeks'), and matched a control group.

Results Patients in the preterm delivery group had a significantly higher incidence of intrauterine growth restriction, lower gestational age at the time of delivery, and lower infant birth weight compared to the other two groups. There was a negative correlation between the sFLT-1/PlGF ratio and GA and between the sFLT-1/PlGF ratio and birth weight at the time of delivery. The value of the sFLT-1/PlGF ratio was significantly higher in the preterm delivery PE group. All the PE group pregnancies ended with caesarean delivery compared to 25% in the control group. However, none of the patients from the PE group had any of the possible complications of preeclampsia nor did they require additional therapy such as blood transfusion or additional non-standard hypertensive therapy.

Conclusion The sFLT-1/PlGF ratio could be used as an indicator for the development and estimation of the severity of PE to provide objective evidence for the management of preeclampsia patients, and as a predictive marker of preeclampsia at low cost.

Key words: eclampsia, pregnancy complications, placenta, premature birth

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INTRODUCTION

Preeclampsia (PE) is a heterogeneous, multisystem disorder, which affects both the mother and the unborn child. It affects approximately 3% of pregnant women, along with other hypertensive disorders it affects 5-10% of all pregnancies and remains one of the leading causes of maternal and perinatal morbidity and mortality (1). In the absence of proteinuria, preeclampsia is diagnosed as hypertension in association with thrombocytopenia (platelet count less than 100,000/microliter), impaired liver function (transaminases up to twice of the normal concentration), new development of renal insufficiency (serum creatinine greater than 1.1 mg/dL or a doubling of serum creatinine in the absence of other renal disease), pulmonary oedema or new-onset cerebral or visual disturbances. Proteinuria is described as excretion of 300 g or more of protein in 24 hour urine collection (2).

The clinical presentation and course of PE is variable, ranging from mild forms, severe and rapidly progressing PE with the need to end the pregnancy and deliver a preterm baby (3). Consequently, preeclampsia is associated with a high risk of iatrogenic preterm delivery, intrauterine growth restriction, placental abruption and perinatal mortality, along with maternal morbidity and mortality (4).

The pathogenesis of PE is complex, and it likely involves maternal, foetal and placental factors. Abnormalities in the development of placental vasculature early in the pregnancy may result in relative placental underperfusion, hypoxia and ischemia, which then lead to release of angiogenic factors into maternal circulation that alter maternal systemic endothelial function and cause hypertension and other manifestations of the disease (5). It is clear that defects in spiral artery remodelling and trophoblast invasion are two separate but related processes characteristic of hypertensive disorder of pregnancy and foetal growth restriction (6). There is growing evidence that imbalance in circulating angiogenic factors, such as placental growth factor (PIGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) secreted by the placenta, may cause endothelial dysfunction, and may have a major role in the development of PE (7). Maynard et al. (8) demonstrated that high serum levels of sFlt-1 (anti-angiogenic protein) and low levels of PIGF (pro-angiogenic protein), predict subsequent development of PE. Studies have demonstrated that a high ratio of sFlt-1/PIGF is linked with PE before its clinical onset, and improves the sensitivity and specificity of Doppler ultrasound in predicting PE (9).

Herraiz et al. (10) proposed an algorithm for the intensive follow-up of patients at risk of developing placental dysfunction-related disorders, based on the rational use of the sFlt-1 to PlGF ratio. As in our study, the first measurement of the sFlt-1 to PlGF ratio was undertaken at 24-28 weeks' gestation. On the basis of the sFlt-1 to PlGF ratio values, they suggested that if the sFlt-1 to PlGF ratio is under the "rule out" cut off point (<38) there is a low risk of developing any early-onset PE and women can follow their regular follow up schedule. If the sFlt-1 to PlGF ratio is in the intermediate range (38-85), those women are highly likely to develop clinical manifestations of PE within four weeks. In this case, a new determination of sFlt-1 to PlGF ratio is needed two weeks later and if the sFlt-1 to PlGF ratio is less than 85 this is considered to be safe from pregnancy complications. In case of an sFlt-1 to PlGF ratio above the "rule out" cut-off point (>85) or symptomatic PE the patients should be considered as having placental dysfunction and be managed according to the current guidelines adding regular reappraisals of the sFlt-1 to PlGF ratio every 48-96h for support of clinical management. Similar recommendations for sFlt-1/PlGF ratio use in the clinical diagnosis of PE are given in the NICE Diagnostic Guidance published in 2016. An sFlt-1/PlGF ratio <38 rules out PE for at least a week, a ratio above 85 (early onset PE) or above 110 (late onset PE) is highly indicative of PE, and a ratio of 38-85 (early onset PE) or 38-110 (late onset PE) provides extra information as to which women are at moderate or high risk of developing PE within four weeks using the sFlt-1 to PlGF ratio (11).

Currently, the diagnosis of PE still mainly relies on clinical signs and symptoms, which are variable and non-specific (hypertension, headache, visual disturbance, epigastric pain, reduced foetal movements and a small infant for the gestational age) (12). As a result, frequent laboratory testing (proteinuria, platelet counts, serum uric acid and liver enzymes levels) is usually required with the assessment of foetal wellbeing and unnecessary hospitalization (13). Although no preventive or therapeutic strategy is yet available, quick and reliable detection of the disease allows imme-

diate intervention with steroids for foetal lung maturity, magnesium for seizure prophylaxis and antihypertensive therapy (14).

Therefore, there is still a need for reliable predictors of PE.

The objective of this study was to investigate the value of using the sFlt-1/PIGF ratio for prediction of the presence or absence of PE, and its influence on clinical validation (decision-making for women with suspected PE in routine clinical practice) and to demonstrate the results with presentation of the cost of the test.

PATIENTS AND METHODS

Patients and study design

A cohort and randomised study was performed at the Clinical Department of Gynaecology and Obstetrics, University Hospital Centre, Osijek, and at the Department of Medical Chemistry, Biochemistry and Clinical Chemistry, University Hospital Centre Osijek, Croatia, between January 2014 and May 2017.

Osijek University Hospital is a tertiary centre and a teaching hospital for an area covering ¼ of the Croatian territory, and the centre responsible for "in utero transport" for Eastern Croatia.

The course of pregnancy was observed extensively in randomly selected pregnant women. Personal and history data, personal habits, the course and pregnancy outcome were recorded.

All patients were presented with and signed an informed consent.

This study was approved by the Ethics Committee of the University Hospital Centre, Osijek.

According to the final pregnancy outcome the pregnant women were divided into two groups: the control group (CTR) and the preeclampsia (PE) group. The control group was selected from women who were normotensive and without proteinuria throughout pregnancy. The diagnostic criteria for PE group were defined as hypertension (systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg after 20 weeks of gestation) and proteinuria with excretion of 300 mg of protein in 24-hour urine. The study at the beginning included 50 pregnant women, however, only 35 participants were followed to the end of the study.

Methods

Maternal blood was collected twice at 24^{+1} to 28^{+0} weeks of gestation (2^{nd} trimester) and 28^{+1} to 32^{+0} weeks of gestation (3^{rd} trimester) in serum vacutainer tubes, and immediately centrifuged at 3500 rpm for 10 min. Serum aliquots were stored at -20°C until analysis, which was done in batches of ten samples according to the availability of the test. Levels of sFlt-1 and PIGF in serum samples were determined retrospectively by means of fully automated Elecsys assays on an electrochemiluminescence immunoassay platform (Cobase analyzers, Roche Diagnostics Ltd. Mannheim, Germany) and used to calculate the sFlt-1/PIGF ratio. The minimal detectable levels in the assays of sFlt-1 and PIGF were 15 and 10 pg/mL. The within-run coefficient of variation for the control samples was below 4% in both assays. Between-run coefficients of variation were 2.3 to 5.6 % for sFlt-1 and 2.4-4.6% for PIGF.

Finally, a total of 35 pregnant women, 12 with preeclampsia (PE) diagnosis and 23 control women (CTR), matched for maternal age and gestational age (GA) at the time of blood sampling, were included into sampling and finished the study. The PE group was subdivided into preterm delivery PE (<37 GA) and term delivery PE (>37 GA). Fifteen women were excluded because they did not attend the second blood sampling (n=13) or missing of delivery data (n=2).

Statistical analysis

Continuous variables were reported as median and categorical variables as numbers (percentage). The normal distribution of the continuous variables was analysed by the Kolmogorov-Smirnov test. Comparison between the defined outcome groups was carried out by the Fisher exact test (categorical variables), Student's t-test (ANOVA) (normal variables), and Mann-Whitney U-test (not normally distributed variables). Differences were considered statistically significant at $p<0.05$. The Spearman correlation coefficient was used to calculate the correlation.

RESULTS

During the study period between January 2014 and May 2017, 50 women were enrolled. Fifteen women were excluded because they did not attend the second blood sampling (n=13) or there was a lack of delivery data (n=2).

There were no differences in age, body mass index (BMI), smoking habits and family history of PE, PE in previous pregnancies or gestational diabetes between the groups. None of the women had pre-existing hypertension. Five (41.7%) women in the PE group and nine (39.1 %) in the control group were nulliparous (Table 1).

Table 1. Demographic and clinical characteristics of the study population at sampling

Characteristic	No (%) of women in the group		p
	CTR (n=23)	PE (n =12)	
Age (years) (range)	32 (21-41)	31.5 (24-35)	0.238
BMI (kg/m2) (range)	27.2 (23-41.5)	29.15 (23-36.4)	0.849
Nulliparous	9 (39.1)	5 (41.7)	
Gestational age (week)* (range)	26 (25-28)	26 (25-28)	
Gestational age (week)† (range)	30 (28-32)	29 (29-32)	
Smoking			
Past	8 (38.1)	5 (41.7)	
Current	4 (19)	2 (16.6)	0.975
No	9 (42.9)	5 (41.7)	
Chronic diseases			
Yes	4 (17.4)	1 (8.3)	0.639
No	19 (82.6)	11 (91.7)	
Drugs			
Metildopa	2 (8.7)	4 (33.3)	
Others	5 (21.7)	0	0.0668
No	16 (79.6)	8 (66.7)	
Family history of PE			
Yes	4 (17.4)	5 (41.7)	0.456
No	19 (82.6)	7 (58.3)	
PE in previous pregnancies			
Yes	5 (21.7)	7 (58.3)	0.322
No	18 (78.3)	5 (41.7)	
GDM in previous pregnancies			
Yes	0 (0)	1 (8.3)	0.343
No	23 (100)	11 (91.7)	

*second trimester ($24^{1/7}$-$28^{0/7}$); †third trimester ($28^{1/7}$-$32^{0/7}$); CTR, matched controls; PE, preeclampsia; BMI, body mass index; GDM, gestational diabetes;

According to the gestational age at delivery six women in the preeclampsia group had preterm delivery (<37 GA) and six women with PE had term delivery (>37 GA). Women in the preterm PE group had a significantly higher incidence of intrauterine growth restriction, with a significantly lower Apgar score and infant birth weight compared to the other two groups (all p <0.001). All the PE group pregnancies were terminated by Caesarean section, compared to 25% in the control group (p<0.001) (Table 2).

The median serum level of sFLT-1 was significantly higher in the preeclampsia group compared to the women in the control group in both trimesters: 2205 *vs* 1309 (p=0.047) in the 2nd trimester, and 3024.5 *vs* 1348 (p=0.004) in the 3rd trimester. In contrast, the median serum level of PlGF was significantly lower in the preeclampsia (PE) group compared to women in the control group in both trimesters: 218.4 *vs* 478.5 (p=0.006) in the 2nd trimester and 151.05 *vs* 570 (p=0.007) in the 3rd trimester. In women with PE, the median sFLT/PlGF ratio was significantly higher compared to the control group in both trimesters: 9.266 *vs* 2.618 (p=0.004) in the 2nd trimester and 25.97 *vs*2.599 (p<0.001) in the 3rd trimester (Table 3).

Table 2. Characteristics of the study population at delivery

	No (%) of women in the group			p
	CTR (n=23)	PE > 37 GA (n=6)	PE < 37 GA (n=6)	
IUGR				
Yes	0	1 (16.7)	4 (66.7)	<0.001
No	23 (100)	5 (83.3)	2 (33.3)	
APGAR	10 (6-10)	10 (10-10)	7 (5-9)	<0.001
Birth weight (g)	3350 (2300-4400)	3230 (2400-4420)	1095 (810-2060)	<0.001
Gestational age (weeks)	39 (36-41)	39 (37-40)	31 (29-36)	<0.001
Delivery				
Vaginal	17 (73.9)	0	0	<0.001
Caesarean section	6 (26.1)	6 (100)	6 (100)	

CTR, matched controls; PE, preeclampsia; GA, gestational age; IUGR, Intrauterine growth retardation; Appearance, APGAR, Pulse, Grimace, Activity, Respiration, score

Table 3. Maternal serum concentration of soluble fms-like tyrosine kinase 1 (sFLT), placental growth factor (PlGF) and sFLT/PlGF ratio in women with preeclampsia and matched controls

	CTR (n=23)	PE (n = 12)	p
sFLT (pg/ml) 24-28 GA	1309 (376.6-2634)	2205 (361-4787)	0.047
sFLT(pg/ml) 28-31 GA	1348 (550-2789)	3024.5 (394.1-6776)	0.004
PlGF (pg/ml) 24-28 GA	478.5 (135.1-1921)	218.4 (29.2-582.1)	0.006
PlGF (pg/ml) 28-32 GA	570 (135-1921)	151.05 (15.5-1039)	0.007
sFLT/PLGF 24-28	2.618 (1.087-6.180)	9.266 (0.958-155.092)	0.004
sFLT/PLGF 28-31	2.599 (0.808-17.890)	25.97 (0.576-217.457)	0.001

CTR, matched controls; PE, preeclampsia; GA, gestational age

Serum levels of PlGF, sFLT-1 and sFLT/PlGF ratio are compared according to the gestational age at delivery, in term and preterm PE. Serum sFLT-1 and sFLT/PlGF ratios were significantly higher in the preterm PE group comparing to term delivery PE women in both trimesters (all p<0.001), and PlGF levels were significantly lower in pre-

term PE women than in term delivery PE women in both trimesters (p=0.014 in the 2nd trimester; p=0.012 in the 3rd trimester). The median, sFLT/PlGF ratio in preterm PE women was significantly higher compared to term delivery PE women in both trimesters (all p<0.001) (Table 4).

Table 4. Comparison of soluble fms-like tyrosine kinase 1 (sFLT), placental growth factor (PlGF) and sFLT/PlGF ratio in term and preterm preeclampsia

	PE >37 GA (n = 6)	PE < 37 GA (n=6)	p
sFLT (pg/ml) 24-28 GA	741.5 (361-2868)	3026,5 (2169-4787)	<0.001
sFLT(pg/ml) 28-31 GA	859.6 (394.1-3045)	5000 (2143-6776)	<0.001
PlGF (pg/ml) 24-28 GA	306.8 (195.6-582.1)	59.7 (29.2-241.3)	0.014
PlGF (pg/ml) 28-32 GA	287.4 (168.2-1039)	46.5 (15.5-133.9)	0.012
sFLT/PLGF 24-28	2.330 (0.958-9.544)	46.643 (8.989-155.092)	<0.001
sFLT/PLGF 28-31	3.375 (0.576-18.103)	123.193 (33.831-217.457)	<0.001

CTR, matched controls; PE, preeclampsia; GA, gestational age

There was a strong, significant negative correlation between the sFLT/PlGF ratio and gestational age (-0.679; p<0.001) and a moderate negative correlation between sFLT/PlGF ratio and birth weight (-0.505; p=0.003).

DISCUSSION

This study supports the theory that preeclampsia results from an imbalance between placental angiogenic and antiangiogenic factors that harm maternal vascular endothelium, resulting in the clinical features of this condition (15). Serum levels of sFlt-1 were significantly higher and PlGF significantly lower in women who developed preeclampsia comparing to women who had a normal pregnancy outcome. The ratio of sFlt-1 to PlGF was also significantly higher in women who developed preeclampsia when compared with women who had a normal pregnancy outcome. Furthermore, serum sFLT-1 and the sFLT/PlGF ratio were significantly higher in the preterm delivery PE group than in the term delivery PE women in both trimesters, and the PlGF level was significantly lower in preterm delivery PE women than in the term delivery PE women, in both trimesters. There was also a statistically significant correlation between the sFlt/PlGF ratio and the week of gestation at delivery, as well as the sFlt/PlGF ratio and birth weight. Women with higher ratios had premature labour and lower infant birth weight.

The number of caesarean sections among preterm labours was significantly higher, as expected. However, the outcome of the pregnancies and the fact that all the new-borns from the PE group were released home with good perinatal outcomes is encouraging.

In a meta-analysis of clinical studies undertaken in the early period of gestation, Kleinrouweler et al. concluded that the test accuracy of PlGF, sFLT1 and sENG was too poor in terms of sensitivity and specificity for accurate prediction of PE in clinical practice (19), but different studies have shown that calculating the sFlt-1/PlGF ratio improves sensitivity for prediction of PE risk (16).

PreOS was the first study to demonstrate the impact of angiogenic biomarkers (in this cases, sFlt1/PlGF) on physicians' clinical decision-making regarding pregnant women with suspicion of preeclampsia in a routine clinical setting. The study also shows that the sFlt-1/PlGF ratio has the potential to be implemented in clinical practice to guide appropriate management intensity (17).

Recent studies have focused on investigations to identify the subgroup that will develop severe PE requiring delivery within the subsequent 1-4 weeks. In high-risk pregnancies, measurement of serum PlGF or the sFlt-1 to PlGF ratio are highly accurate in identifying the target group (18-20). Close monitoring of such pregnancies for earlier diagnosis of clinical signs of the disease could potentially improve perinatal outcome through interventions such as antihypertensive therapy and early delivery (21).

Although there was a relatively small number of participants, our results suggest that measurement of serum sFlt-1 and PlGF or the sFlt-1 to PlGF ratio is highly accurate in identifying not only the development of severe PE but also women who will have premature labour. Women with PE in our study who had premature labour, lower infant birth weight and low Apgar score had significantly higher sFlt-1 values and sFlt-1 to PlGF ratio than PE women with term delivery and the control group, in both trimesters. PlGF values were significantly lower in women with PE who had premature labour, lower infant birth weight and low Apgar score, than PE women

with term delivery and the control group, in both trimesters.

The results of our study are within the framework of the proposed algorithm and NICE guidelines, considering the sFlt-1/PlGF ratio cut-off and the prediction period. Women with an sFlt-1/PlGF ratio >38 in the 2nd trimester and >85 in the 3rd trimester developed PE and had preterm delivery within the subsequent 4 weeks.

Despite the ambiguous results from different studies, the sFlt-1 to PlGF ratio could be useful test for identifying potential risk groups among pregnant women with PE symptoms. In addition, the equipment already exists in small hospitals and community health centres (such as in this case electrochemiluminescence immunoassay, ECLIA Cobase 411 analyzer - Roche Diagnostics GmbH Mannheim, Germany, 2014) and testing equipment could be used to determine possible cases that should be sent to the appropriate hospital as "in utero transport" increasing the viability of children and reducing maternal morbidity and mortality.

The cost of sFlt/PlGF testing is approximately 40 Euros (300 HRK), which is acceptable when comparing the cost of usual medications used in the treatment of eclampsia (e.g. the price of a single dose (300 mL) of full blood is 500 HRK; the price of basic single-day intravenous antihypertensive therapy with ebrantil (urapidil) is 260 HRK (approx. 34 Euros), with carboprost or mifepriston therapy the price ranges between 150 HRK and 250 HRK (20 and 33 Euros, respectively). The value of lower maternal mortality and morbidity, and the value of low perinatal mortality also add to the arguments for introducing sFlt/PlGF testing.

Appropriate screening, monitoring and routine check-ups during pregnancy may prevent the deterioration of the maternal and foetal condition (15,16).

The results of our study suggest that the sFlt-1/PlGF ratio could be used as an indicator even in smaller hospitals, in countries with a low to moderately well-developed health system, and to serve as a potential tool for the diagnostics and management of patients with preeclampsia.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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