

Fibronectin, plasminogen activator inhibitor type 1 (PAI-1) and uterine artery Doppler velocimetry as markers of preeclampsia

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Abstract. *Objective:* The purpose of this study was to examine plasma levels of fibronectin and plasminogen inhibitor type 1 (PAI-1), and alterations in uterine artery (UtA) waveforms throughout normotensive and preeclamptic pregnancies and to analyze its predictive value for the detection of preeclampsia within the second trimester of pregnancy.

Material and methods: Blood samples were collected from 102 healthy, nulliparous women between the 24th and 26th gestational week. Preeclampsia developed in 13 patients; 89 normotensive control subjects were matched from the same cohort. Plasma samples were assayed for fibronectin and PAI-1 by enzyme-linked immunosorbent assay. Color pulsed Doppler examinations of UtA were performed after blood sampling. Trends were compared between two groups.

Results: Maternal plasma fibronectin and PAI-1 levels and average PI, RI and S/D ratios of patients with preeclampsia were significantly higher ($p < 0.05$). The best cut-off values for predicting preeclampsia of fibronectin, PAI-1, PI, RI, S/D ratio based on ROC curve analysis were 290 mg/ml, 77.3 ng/ml, 1.0615, 0.605 and 2.59 respectively. The areas under the curve equal to 0.705, 0.753, 0.689, 0.695 and 0.699 for fibronectin, PAI-1 and uterine artery Doppler PI, RI, S/D ratio were determined for the prediction of preeclampsia.

Conclusions: Fibronectin, PAI-1 and UtA Doppler are potentially useful predictors of preeclampsia. Maternal plasma PAI-1 combined with fibronectin had the highest predictive values in our study.

Keywords: Preeclampsia, gestational hypertension, fibronectin, PAI-1, uterine artery Doppler

1. Introduction

Preeclampsia is a multisystem disorder specific to pregnant women. It is characterized by maternal hypertension, proteinuria, edema, potentially causing fetal growth restriction and premature delivery [1]. This common pregnancy-associated disease is a leading cause of perinatal morbidity and mortality of mother and child [2]. Although the exact pathogenesis of preeclampsia still remains to be unclear, several mech-

anisms, varying from immune maladaptation to gene expression, have been proposed. It becomes increasingly clear, that pathological processes at the interface of the fetal and maternal circulation lead to generalized endothelial cell dysfunction [3,4].

Maternal endothelial dysfunction could be mediated by factors present in the maternal circulation as a result of abnormal placentation and impaired trophoblast invasion of maternal spiral arteries during early gestation [4–6]. The signs and symptoms of the disease are well recognized and they generally manifest in the second to third trimester, although the underlying pathology is present at earlier stages of pregnancy. The identification of reliable screening markers could predict the onset of preeclampsia before clinical manifestations. This would permit improvements in obstet-

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ric care through better targeting of antepartum surveillance, identification of high risk pregnancies that may benefit from early therapeutic interventions or potential prophylactic treatments such as antioxidants.

The aim of this study was to assess whether alterations in the maternal plasma levels of fibronectin and PAI-1 could be detected in patients before clinical manifestation of disease, and to evaluate validity of these markers and uterine artery (UtA) Doppler velocimetry in predicting preeclampsia.

2. Materials and methods

2.1. Study design

The cross-sectional longitudinal case-control study was set at the Department of Gynecology and Obstetrics, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia (JFM CU). The protocol was approved by the Ethical Committee JFM CU. All participants signed informed consent.

2.2. Study population and methods

We enrolled 102 singleton pregnancies, which were divided into two groups (control: $n = 89$, preeclampsia: $n = 13$). Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, after 20th gestational week (gw). Preeclampsia was diagnosed in the presence of hypertension (two separate readings taken at least 4 hours apart of 140/90 or more) and proteinuria (≥ 300 mg in a 24 hours urine collection, or 2 dipstick measurement of 1 cross or 1 dipstick measurement ≥ 2 crosses using PentaPhan, PLIVA-Lachema Diagnostika, Brno, Czech republic), according to ACOG [2].

Single maternal venous blood samples (cubital vein), obtained between the 24th and 26th gw at the time of routine screening test (oral glucose tolerance test), were collected into Vacutainer[®] tubes with 5% EDTA. Plasma samples were isolated by 2,500g centrifugation for 10 min. and stored at -40°C . Maternal plasma fibronectin (FBN) a plasminogen activator inhibitor type 1 (PAI-1) levels were determined by enzyme-linked immunosorbent assay (Asserachrom PAI-1, Diagnostica Stago, Gennevilliers, France; ZYMUTEST Fibronectin, HYPHEN BioMed, Neuville sur Oise, France).

All color pulsed Doppler examinations of UtA were performed by the first author and supervised by expe-

rienced sonographer. We used ALOKA Prosound $\alpha 10$ with a 3.5 MHz abdominal probe (ALOKA, Tokyo, Japan). UtA were identified in the lower lateral quadrant of the uterus, 1 cm above the crossing point of external iliac artery and UtA. High-pass filter was set at 100 Hz. Doppler waveform was obtained from both UtA. When three uniform consecutive waveforms were obtained, the pulsatility index (PI), resistance index (RI) and S/D ratio (S – peak systolic velocity; D – peak diastolic velocity) were measured. The mean value of three waveforms was used for subsequent analysis. The laboratory staff performing the assays was blinded to the clinical information.

2.3. Statistical analysis

The normality of the data was tested using the Kolmogorov-Smirnov test. Student's t-test was used for comparison of two groups, using statistical software Medcalc 10.2, Mariakerke, Belgium. A receiver-operator characteristic (ROC) curves were constructed. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) in the prediction of preeclampsia were calculated. A p-value < 0.05 was considered significant.

3. Results

In Table 1, the clinical characteristics of the patient populations are shown. From the 102 pregnancies, 13 developed preeclampsia. No difference was found in the average maternal age. As expected, weight before pregnancy and body mass index was significantly higher in preeclamptic patients. In group of preeclamptic patient we found higher number of primigravidas. Neonatal birth weight was significantly higher in unaffected pregnant women.

Maternal plasma fibronectin and PAI-1 levels and average PI, RI and S/D ratios of preeclamptic patients were significantly higher (Table 2). The sensitivities and false-positive rates (ROC curves) for the detection of preeclampsia by measurement of maternal plasma fibronectin, PAI-1 concentrations and abnormal UtA velocimetry are shown in Table 3 and Fig. 1. The areas under the curve equal to 0.705, 0.753, 0.689, 0.695 and 0.699 for fibronectin, PAI-1 and UtA Doppler PI, RI, S/D ratio were determined for the prediction of preeclampsia. The best cut-off values for predicting preeclampsia of fibronectin, PAI-1, PI, RI, S/D ratio based on ROC curve analysis were 290 mg/ml, 77.3 ng/ml, 1.0615, 0.605 and 2.59 respectively. Screening characteristics for preeclampsia by combination of two markers are shown in Table 4.

Table 1
Clinical characteristics of women who later developed preeclampsia and control group (data are presented as mean \pm standard deviation)

	Controls (<i>n</i> = 89)	Preeclampsia (<i>n</i> = 13)	p
Age (years)	30.2 \pm 5.27	29.47 \pm 4.26	NS
Body Mass Index	22.43 \pm 3.77	25.74 \pm 6.33	< 0.05
Primigravidas	23.2%	47.1%	< 0.05
Birth weight (g)	3393.13 \pm 509.95	3106.67 \pm 809.85	< 0.05
Gestational age	40.04 \pm 2.47	38.40 \pm 2.32	< 0.05
IUGR	1.1%	7.7%	< 0.05
Apgar score 1 minute	8.91 \pm 0.94	8.9 \pm 0.32	NS
Apgar score 5 minute	9.12 \pm 0.75	9.0 \pm 0.47	NS

Table 2
Maternal plasma fibronectin and PAI-1 levels, and PI, RI and S/D ratio in UtA (data are presented as mean \pm standard deviation)

	Controls (<i>n</i> = 89)	Preeclampsia (<i>n</i> = 13)	p
Fibronectin (mg/ml)	337.75 \pm 120.74	427.19 \pm 101.76	< 0.05
PAI-1 (ng/ml)	73.00 \pm 25.30	102.68 \pm 33.25	< 0.05
PI	0.83 \pm 0.35	1.17 \pm 0.61	< 0.05
RI	0.51 \pm 0.10	0.6 \pm 0.15	< 0.05
S/D	2.19 \pm 0.80	3.17 \pm 2.00	< 0.05

Table 3
Screening characteristics of markers above cut-off values (PPV- positive predictive value, NPV- negative predictive value)

	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Fibronectin	> 290.00	92.3	42.6	43.64	91.99
PAI-1	> 77.30	84.6	66.7	43.97	93.34
PI	> 1.0615	53.8	86.0	35.86	92.75
RI	> 0.605	53.8	86.0	35.86	92.75
S/D	> 2.59	53.8	86.5	36.07	92.79

4. Discussion

Poor placental perfusion and other pathologic changes in the placenta such as defective trophoblast invasion of spiral arteries characterise preeclampsia and form the basis for examination of endothelial plasma markers such as fibronectin and PAI-1.

Fibronectin belongs to a family of large glycoproteins (440–500 kDa) [7]. Fibronectin plays also major role in cell adhesion, growth, migration and differentiation, and it is important for processes such as wound healing and embryonic development [8]. It is synthesized in the endothelial cells and its elevated level may be an indicator of endothelial damage. Total fibronectin makes up the majority of fibronectin present in the circulation. In different studies, both total and cellular fibronectin levels were found to be elevated prior to onset of preeclampsia [9–12]. In the present study increased second trimester plasma levels of fibronectin in patients who later developed preeclampsia has been observed.

Our findings were in agreement with those of Dane et al., Aydin et al. who reported that preeclampsia is associated with an increased plasma fibronectin level compared to that of normal pregnancies [9,10]. The association of elevated plasma fibronectin in preeclampsia has been documented in previous studies indicating an endothelial cell dysfunction [12]. In the study of Gredmark et al., the positive predictive value in 26th gw was 57%, the specificity 97%, whereas the sensitivity was only 17% [11]. The authors concluded that total fibronectin might be useful to determine relative risks, but is a poor predictor in a low risk population. Tjoa et al. repeated this plasma analysis of total fibronectin in a similar low risk population, with emphasis on the first trimester and found no differences in plasma total fibronectin levels in pregnant women between 7th-20th gw [1]. Ostlund et al. showed relation of plasma total fibronectin to blood pressure in women who developed preeclampsia in 16 gw and even higher in those with laboratory signs of organ involvement [7]. They found positive correlation among fibronectin and PAI-

Table 4
Screening characteristics of markers combination above cut-off values from Table 3 (PPV- positive predictive value, NPV- negative predictive value, AUC – area under curve)

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC
FBN + PAI-1	90.90	79.10	34.26	98.64	0.908
FBN + PI	90.91	79.67	35.12	98.64	0.885
FBN + RI	81.82	79.12	32.18	97.29	0.864
FBN + S/D	81.82	79.12	32.18	97.29	0.864
PAI-1 + PI	81.82	82.42	36.04	97.40	0.869
PAI-1 + RI	81.82	80.22	33.37	97.33	0.869
PAI-1 + S/D	81.82	80.22	33.37	97.33	0.869

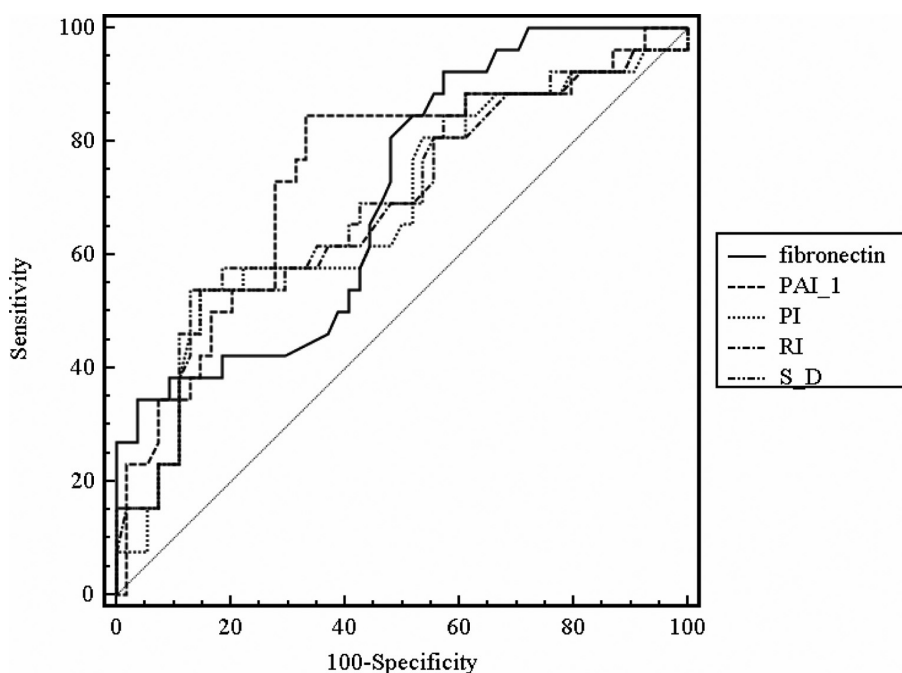


Fig. 1. Receiver operating characteristic curves showing the prediction performance of fibronectin, PAI-1 and UtA Doppler PI, RI, S/D ratio.

1. Increased plasma fibronectin levels, even before the clinical onset of preeclampsia, support the concept of preeclampsia as an endothelial cell dysfunction disease.

Plasminogen activator inhibitor type 1 (PAI-1) is a principle regulator of fibrinolysis and a marker of endothelial dysfunction [13]. It is not only produced by the endothelial cells, but also activated platelets, placental vasculature and trophoblasts [3]. Its increased levels can be caused by PAI-1 hyperproduction in various compartments including visceral adipocytes and hepatocytes [14,15]. PAI-1 rises in a variety of disorders characterized by hypertension and renal damage. An increased PAI-1 concentration promotes release of platelet-derived growth factors, which are known to play an important role in vascular injury [3]. PAI-1 can be used as indirect marker of endothelial function and may also reflect platelet activation and malplac-

tation, known to associate with the severity of the disease. This is in the line of Catarino et al. work, who found significant positive correlation between maternal PAI-1 levels and proteinuria [16]. PAI-1 plasma levels in our study were found to be significantly higher in preeclampsia compared to levels of healthy controls. In recent work, Swellan et al. also found increase levels of PAI-1 with sensitivity of prediction 98%, and even higher with combination of PAI-1 and C-reactive protein (CRP) [13]. They attributed the hypothesis that hypoxia may be responsible for increased syncytial PAI-1 expression noted in preeclampsia, which is suggested to promote increased intervillous fibrin deposition that is observed in these pregnancies [13,17].

Combination of UtA Doppler findings, namely bilateral uterine notching, was performed by Hunt. et al. In their study increased PAI-1 levels were associated

with bilateral notching in 23rd gw [18]. In contrast, Djurovic et al. showed no differences in plasma concentration of PAI-1 at 18th gw between women who subsequently developed preeclampsia and matched controls women [19]. PAI-1 exist in non-pregnant individuals in significant quantities in both plasma and platelets and its plasma levels are altered in a variety of diseases where platelets number tend to vary. Because of the involvement of PAI-1 in various pathophysiologic processes, such as impaired endothelial and placental function and platelet activation, PAI-1 might be a nonspecific marker of preeclampsia. The rise of PAI-1 noted in preeclampsia may reflect a nonspecific response of this type; alternatively it could reflect placental damage.

Doppler ultrasound enables the assessment of blood flow parameters for the adequate and reduced perfusion *in vivo*. Impaired trophoblastic invasion of the maternal spiral arteries is shown to be associated with increased impedance to flow in the waveforms obtained by Doppler ultrasound examination of the UtA [20,21]. Bilateral high-resistance flow velocity waveforms with early diastolic notches at 22nd–26th gw are associated with subsequent fetal death, intrauterine growth restriction (IUGR) and preeclampsia [22]. High mean PI, RI and S/D ratios in UtA have also been demonstrated in pregnancies destined to be preeclamptic by the present study. The conversion of spiral arteries into uteroplacental arteries plays a basic role in the establishment of the physiological placental blood supply. The reduced uteroplacental blood flow due to inadequate trophoblastic infiltration of the placental vascular bed can be the cause of a variety of pregnancy complications, such as IUGR, eclampsia or HELLP syndrom.

Based on ROC curves in the current study fibronectin, PAI-1 and UtA Doppler were all potentially clinically useful tests for prediction of preeclampsia. Maternal plasma PAI-1 had the highest predictive value (AUC = 0.753) for early identification of women at increased risk for the development of preeclampsia. Fibronectin and UtA Doppler was found to be slightly less.

Plasma fibronectin, PAI-1 and UtA Doppler PI, RI and S/D ratio were found to detect women at increased risk of preeclampsia with a sensitivity of 92%, 85%, 54%, 54% and 54% and a specificity of 43%, 67%, 86%, 86% and 86,5% respectively. Because of the low values of the specificity, we decided to improve sensitivity and specificity of tests by their combination. In second trimester, best predictive markers combination resulting from our results, would be PAI-1 together with fibronectin (AUC = 0.908), followed by combination of fibronectin and UtA PI (AUC = 0.885).

The incidence of preeclampsia detected in our group is higher than the 5-7% prevalence of preeclampsia in the normal population. The screening performance of the tests may be high with the high prevalence of preeclampsia in our study group.

The limitations of this study include its small size. Our study did not include any women who developed post-partum PEE or eclampsia or who developed a worsening clinical outlook after delivery.

5. Conclusion

We demonstrated potentially effective and clinically applicable predicting tests for preeclampsia, at least in a high-risk population. Fibronectin, PAI-1 and UtA Doppler are potentially useful predictors of preeclampsia. Maternal plasma PAI-1 combined with fibronectin had the highest predictive values in our study.

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Disclosure of interest

The authors report no conflicts of interest. The authors are responsible for content and writing of the paper.

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