

ORIGINAL ARTICLE

Markers of Endothelial Activation in Preeclampsia

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SUMMARY

Background: The study aimed at finding a laboratory approach to detect endothelial damage in normal pregnancy as well as in pregnancy complicated by preeclampsia using selected markers of endothelial activation.

Materials: A total of 403 healthy pregnant women without a history of deep vein thrombosis and/or hypertension were prospectively studied. From all women, venous blood was collected before the end of the 1st trimester, between weeks 24 and 28 of gestation, and in the 3rd trimester (weeks 33-36). Assays of tissue plasminogen activator, plasminogen activator inhibitor-1, von Willebrand factor activity and antigen, thrombomodulin, endothelial protein C receptor, and endothelial microparticles activated by TF were performed.

Results: When comparing women who developed preeclampsia during pregnancy (the average levels were 23.41 µg/L, 34.33 µg/L, and 53.56 µg/L in the 1st, 2nd, and 3rd trimesters, respectively) with healthy pregnant women (the average levels were 19.05 µg/L, 28.47 µg/L, and 39.86 µg/L in the 1st, 2nd, and 3rd trimesters, respectively) significant differences in the levels of thrombomodulin were found in all three trimesters. By contrast, no statistically significant differences in the levels of vWF (both antigen and activity), PA, EPCR, EMPs, MMP-2, MMP-9, and TIMP-9 were found in any trimesters in the same group.

Conclusions: Pregnancy and preeclampsia strongly influence the levels of studied markers. The findings of this work confirm the possible predictive potential of thrombomodulin and PAI-1.
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KEY WORDS

endothelium, matrix metalloproteinases, coagulation activation markers, pregnancy complications, preeclampsia

LIST OF ABBREVIATIONS

EMPs - endothelial microparticles (particles/µL)

EPCR - endothelial protein C receptor (µg/L)

MMP-2 - matrix metalloproteinase-2 (RFU - relative fluorescence units)

MMP-9 - matrix metalloproteinase-9 (RFU - relative fluorescence units)

PAI-I - plasminogen activator inhibitor-1 (µg/L)

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TIMP-2 - tissue inhibitor of metalloproteinase- 2 (ng/mL)
 TRM - thrombomodulin ($\mu\text{g/L}$)
 t-PA - tissue plasminogen activator ($\mu\text{g/L}$)
 vWF act. - von Willebrand factor activity (%)
 vWF ag. - von Willebrand factor antigen (%)
 U - statistic criterium Mann Whitney test
 Z - pooled selection for Mann Whitney test

INTRODUCTION

Normal pregnancy is accompanied by significant changes in all aspects of hemostasis. The changes include both increased levels of most coagulation factors (I, V, VII, VIII, vWF, IX, X, and XII) as well as decreased levels or activity of certain natural coagulation inhibitors (protein S levels, decreased sensitivity to activated protein C). In addition, the fibrinolytic system is significantly modified (lower t-PA and higher PAI-1 and PAI-2 levels) [1,2].

Hormonal and hemodynamic changes during pregnancy also result in alterations in the function of the endothelium [3,4]. Together with smooth muscle cells and fibroblasts, endothelial cells make up the vascular wall. They are arranged in a single layer along the luminal surface of blood vessels. The cells are not simply a passive lining but are an extremely metabolically active organ providing a barrier between blood and tissues. They may be considered both as sensory structures receiving hemodynamic and humoral signals as well as effector cells producing numerous agents (cytokine receptors, growth factors, bacterial substances, angiotensin II, coagulation factors II, V, IX or X, etc.) [5]. These stimuli activate the endothelium, resulting in a release of various products through which endothelial cells control vasomotor activity (vascular tone, blood pressure, organ perfusion), coagulation (platelet adhesion and aggregation, coagulation cascade and fibrinolysis activation), rheological properties of blood, and the inflammatory response (migration and chemotaxis of leukocytes, monocytes, and macrophages, phagocytosis, vascular permeability) [5,6]. All these events play a key role in the regulation of cellular metabolism.

Abnormal placentation is central to the inception of this disease process. However, the triggering factor of this is still unknown. Interestingly, the intense research done in this area has unveiled biomolecules which play an important role in the vasculogenesis of the early placenta, namely, vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) and their antagonists, namely, soluble fms-like tyrosine kinase 1 (sFlt-1, also known as sVEGFR1), and soluble endoglin (sEng) [7,8]. Fibronectin seems to be a promising marker for the prediction of pre-eclampsia; however, further studies are needed to determine whether the accuracy of this test is sufficient to be clinically relevant.

Clotting disorders are associated with the severe, early, and complicated forms of preeclampsia. The early increase of fibronectin levels, the worsening of the throm-

bocytopenia and the raised platelet turnover are laboratory markers of platelet and endothelial activation. The excessive thrombin formation is physiologically compensated by a rise in thrombin-antithrombin (TAT) complex levels, which is the most specific marker of a preeclampsia pregnancy. The placenta induced depression of fibrinolysis appears to contribute towards the hypercoagulable state with high levels of fibronectin as a next new marker [9].

The results of recent studies demonstrate that fibronectin may be a more valuable biomarker than thrombomodulin and PAI-1 for the assessment of endothelial damage in preeclampsia and eclampsia [10-14].

The study aimed at finding a laboratory approach to detecting endothelial damage during normal pregnancy as well as pregnancy complicated by preeclampsia using certain markers of endothelial activation.

Based on the available recent literature, the study comprised assays of tissue plasminogen activator, plasminogen activator inhibitor-1, von Willebrand factor activity and antigen, thrombomodulin, endothelial protein C receptor, and endothelial microparticles.

MATERIALS AND METHODS

A total of 403 healthy pregnant women without a history of deep vein thrombosis and/or hypertension were prospectively studied. From all women, venous blood was collected before the end of the 1st trimester, between weeks 24 and 28 of gestation, and in the 3rd trimester (weeks 34 - 36). The obtained blood samples were subsequently processed in the coagulation laboratory. For the assays, venous blood was sampled into 0.129 M sodium citrate (Vacuette, Greiner). Platelet-poor plasma (PPP) was obtained by centrifugation at 3000 g for 10 minutes and kept at -80°C until processed. Endothelial microparticles (EMPs) were measured using venous blood samples collected into K_3EDTA . All patients agreed to participate in the study by signing informed consent. The study was approved by the Palacký University Faculty of Medicine and Dentistry Ethics Committee.

During the prospective follow-up of 403 patients, mild, moderate or severe preeclampsia was diagnosed in 39 (9.6%) and HELLP syndrome in 8 (1.9%) pregnant women. Laboratory markers of endothelial activation were subsequently examined and compared in both groups (normal pregnancy vs. preeclampsia) in all trimesters. Inclusion criteria for mild and moderate preeclampsia were as follows: blood pressure $> 140/90$ and $< 160/110$; proteinuria > 300 mg/24 hours. Severe preeclampsia was defined as diastolic blood pressure of at least 110 mm Hg, or systolic blood pressure of at least 160 mm Hg and/or thrombocytopenia $< 100 \times 10^9/\text{L}$, elevation of liver enzymes, and hemolysis with schistocytes.

t-PA and PAI-1

Tissue-type plasminogen activator antigen and plasminogen activator inhibitor-1 levels were determined by the ELISA method (Technoclone GmbH, Austria, CE IVD).

Von Willebrand factor

Von Willebrand factor antigen levels were determined by enzyme immunoassay (Instrumentation Laboratory, Italy, CE IVD).

Soluble thrombomodulin antigen and endothelial protein C receptor

Soluble thrombomodulin antigen and endothelial protein C receptor levels were determined by the ELISA method (Diagnostica Stago, France, RUO).

Endothelial microparticles

EMPs were quantified in plasma as previously described [15]. Thirty μL of platelet-free plasma were incubated for 30 minutes at room temperature with 10 μL of PE-conjugated CD144. The samples were then diluted in 1.0 mL PBS and a known number of fluorescent latex beads (Flowcount, Beckman Coulter Immunotech, France, RUO) were added to samples according to the internal standards before flow cytometry analysis. The EMPs were analyzed using a Coulter Epics XL (Beckman Coulter, Switzerland, Nyon) as previously described [15]. Using 0.8 μm latex beads, EMP were defined as elements less than 1 μm in size and positively labeled with PE-conjugated CD144. The results were expressed as the number of EMPs per 1 mL of plasma.

Matrix metalloproteinases

The MMP-2 and MMP-9 activity in plasma samples was measured by the solid-phase ELISA method¹² with EDANS/DABCYL FRET peptide (AnaSpec, USA, RUO). The results were expressed in reference fluorescence units (RFU). The RFU is a unit of measurement used in ELISA methods employing fluorescence detection. Fluorescence is detected as labeled fragments, conjugated on solid phase, and excited by laser. The software interprets the results, calculating the quantity of the fragments from the fluorescence intensity of each sample.

Tissue inhibitor of metalloproteinase²

The TIMP-2 activity in plasma samples was measured by solid-phase ELISA (R&D Systems, USA, RUO).

Statistical analysis

The statistical analysis and graphical presentation were performed using the statistics software (StatSoft CR s.r.o. (2007). STATISTICA Cz). Clinical and laboratory data are given as means and standard deviation (SD). Differences between trimesters were analyzed using the non-parametric Wilcoxon signed-rank test and the non parametric Friedman ANOVA. Other data were analyzed using the Mann-Whitney and Kruskal-Wallis

tests. The level of significance was set at 5%.

RESULTS

Between 2007 and 2009, blood samples from 403 healthy pregnant women were analyzed. Their medical history was negative. Of the women, 298 (74%) were primiparous, 72 (18%) secundiparous and 33 (8.1%) tertiparous.

Their average age was 27.6 years (\pm 4.5 years). The average maternal weight at the beginning of pregnancy was 62.6 kg (\pm 8.8 kg) and the average weight gain was 10.03 kg (\pm 4.4 kg). The average height of the women was 167 cm (\pm 5.6 cm).

Of the 403 women, 39 (9.6%) developed mild to moderate preeclampsia, eight (1.9%) were diagnosed with HELLP syndrome with clinical manifestations of various severity, 28 (7%) gave birth prematurely, before the end of week 37 of gestation, and in 5 women (1.2%), congenital abnormalities led to a legal abortion in the first or second trimester. Thus, a total of 358 pregnant women participated in the complete protocol of the study, i.e., sampling in all three trimesters.

Laboratory results

Comparing the development of endothelial activation markers in the trimesters

The levels of vWF antigen continued to increase throughout pregnancy (the average levels were 152.32%, 173.34%, and 216.20% in the 1st, 2nd, and 3rd trimesters, respectively). A significant difference was found between the 2nd and 3rd trimesters, with a trend between the 1st and 2nd trimesters. At the same time, vWF activity was on the rise (the average levels were 130.20%, 150.09%, and 181.91% in the 1st, 2nd, and 3rd trimesters, respectively). A significant difference was found between the 2nd and 3rd trimesters but not between the 1st and 2nd trimesters. The levels of thrombomodulin increased significantly during pregnancy (the average levels were 19.05 $\mu\text{g/L}$, 28.47 $\mu\text{g/L}$, and 39.86 $\mu\text{g/L}$ in the 1st, 2nd, and 3rd trimesters, respectively). Statistically significant differences were found both between the 1st and 2nd trimesters and between the 2nd and 3rd trimesters. The levels of soluble EPCR continued to rise throughout pregnancy (the average levels were 201.76 $\mu\text{g/L}$, 274.68 $\mu\text{g/L}$, and 324.07 $\mu\text{g/L}$ in the 1st, 2nd, and 3rd trimesters, respectively). A statistically significant difference was found between the 1st and 2nd trimesters but not between the 2nd and 3rd trimesters. The PAI-1 levels increased during the pregnancy (the average levels were 36.14 $\mu\text{g/L}$, 50.07 $\mu\text{g/L}$, and 60.12 $\mu\text{g/L}$ in the 1st, 2nd, and 3rd trimesters, respectively). Significant differences were found both between the 1st and 2nd trimesters and between the 2nd and 3rd trimesters.

Table 1. Comparing the development of endothelial activation markers in the trimesters.

| Endothelial marker | Mean value-normal pregnancy | Mean value-preeclampsia | Standard deviation - normal pregnancy | Standard deviation - preeclampsia | Comparison of normal pregnancy vs. preeclampsia p = |
|---|-----------------------------|-------------------------|---------------------------------------|-----------------------------------|---|
| vWF act. I (%) | 130.20 | 117.08 | 36.94 | 29.59 | 0.0613 |
| vWF act. II (%) | 150.09 | 142.05 | 49.21 | 33.52 | 0.9031 |
| vWF act. III (%) | 181.91 | 213.00 | 62.31 | 65.40 | 0.0035 |
| vWF ag. I (%) | 152.32 | 159.28 | 45.75 | 48.27 | 0.7431 |
| vWF ag. II (%) | 173.34 | 181.39 | 51.61 | 50.59 | 0.4069 |
| vWF ag. III (%) | 216.20 | 219.03 | 69.66 | 70.76 | 0.8032 |
| TRM I (µg/L) | 19.05 | 23.41 | 9.37 | 11.52 | 0.0467 |
| TRM II (µg/L) | 28.47 | 34.33 | 16.64 | 15.18 | 0.0117 |
| TRM III (µg/L) | 39.86 | 53.56 | 20.39 | 28.62 | 0.0071 |
| EPCR I (µg/L) | 201.76 | 151.26 | 139.02 | 90.72 | 0.0130 |
| EPCR II (µg/L) | 274.68 | 233.74 | 179.90 | 170.48 | 0.1896 |
| EPCR III (µg/L) | 324.07 | 398.69 | 215.54 | 235.45 | 0.0746 |
| PAI-1 I (µg/L) | 36.14 | 36.87 | 18.93 | 16.50 | 0.6141 |
| PAI-1 II (µg/L) | 50.07 | 59.54 | 19.66 | 18.33 | 0.0170 |
| PAI-1 III (µg/L) | 60.12 | 77.03 | 19.89 | 19.84 | 0.0000 |
| EMPs I (particles x10 ⁹ /mL) | 3.838 | 4.161 | 3.176 | 2.781 | 0.0748 |
| EMPs II (particles x10 ⁹ /mL) | 3.836 | 3.082 | 2.061 | 1.374 | 0.0622 |
| EMPs III (particles x10 ⁹ /mL) | 3.630 | 4.238 | 1.441 | 2.559 | 0.4701 |
| t-PA I (µg/L) | 2.48 | 2.59 | 1.09 | 0.88 | 0.4925 |
| t-PA II (µg/L) | 2.97 | 3.33 | 1.46 | 2.70 | 0.9177 |
| t-PA III (µg/L) | 3.34 | 2.92 | 2.12 | 1.06 | 0.5305 |
| MMP-2 I (RFU/mL) | 9043.76 | 9038.39 | 3073.44 | 2530.73 | 0.6664 |
| MMP-2 II (RFU/mL) | 9315.38 | 7419.77 | 2720.44 | 1672.04 | 0.0001 |
| MMP-2III (RFU/mL) | 8800.27 | 8403.28 | 2654.92 | 1470.54 | 0.7040 |
| MMP-9 I (RFU/mL) | 8371.90 | 8278.92 | 3168.46 | 2526.47 | 0.6573 |
| MMP-9 II (RFU/mL) | 8290.81 | 6408.44 | 2373.71 | 1779.78 | 0.0000 |
| MMP-9III (RFU/mL) | 7470.50 | 7100.08 | 2456.88 | 1135.13 | 0.9227 |
| TIMP-2 I (µg/L) | 1.85 | 1.68 | 0.79 | 0.81 | 0.4440 |
| TIMP-2 II (µg/L) | 1.97 | 1.83 | 0.77 | 0.71 | 0.1415 |
| TIMP-2III (µg/L) | 1.93 | 2.06 | 0.74 | 0.72 | 0.2882 |

Comparing the individual parameters in women with preeclampsia and in normal pregnancy (controls) Significant differences of thrombomodulin were found in all three trimesters when comparing women with preeclampsia (the average levels were 23.41 µg/L, 34.33 µg/L, and 53.56 µg/L in the 1st, 2nd, and 3rd trimesters, respectively) and controls - healthy pregnant women (the average levels were 19.05 µg/L, 28.47 µg/L, and 39.86 µg/L in the 1st, 2nd, and 3rd trimesters, respectively).

Significant differences in the levels of PAI-1 were found in the 2nd and 3rd trimesters, but not in the 1st trimester, when comparing women with preeclampsia (the average levels were 36.87 µg/L, 59.54 µg/L, and 77.03 µg/L in the 1st, 2nd, and 3rd trimesters, respectively) and controls - healthy pregnant women (the average levels were 36.14 µg/L, 50.07 µg/L, and 60.12 µg/L in the 1st, 2nd, and 3rd trimesters, respectively). EMPs, MMP-2, MMP-9, and TIMP-9 were found in any trimesters when comparing women with preeclampsia

Table 2. The comparison of endothelial activation markers in controls and in women with preeclampsia.

| Markers of endothelial activation | | | |
|--|--------|--------|--------|
| Mann-Whitney U test, significance level $p < 0.05$ | | | |
| | U | Z | P |
| vWF antigen I | 1521.0 | -0.328 | 0.7431 |
| vWF antigen II | 413.5 | -0.829 | 0.4069 |
| vWF antigen III | 1744.5 | -0.249 | 0.8032 |
| vWF akt. I | 1245.5 | 1.871 | 0.0613 |
| vWF akt. II | 1538.5 | 0.122 | 0.9031 |
| vWF akt. III | 1214.0 | -2.919 | 0.0035 |
| Trombomodulin I | 1129.5 | -1.989 | 0.0467 |
| Trombomodulin II | 966 | -2.522 | 0.0117 |
| Trombomodulin III | 1128.5 | -2.690 | 0.0071 |
| tPA I | 1457 | -0.686 | 0.4925 |
| tPA II | 1464.5 | 0.103 | 0.9177 |
| tPA III | 1596 | 0.627 | 0.5305 |
| PAI-1 I | 1489.5 | -0.504 | 0.6141 |
| PAI-1 II | 1078 | -2.387 | 0.0170 |
| PAI-1 III | 848.5 | -4.534 | 0.0000 |
| EPCR I | 1002 | 2.483 | 0.0130 |
| EPCR II | 1038 | 1.312 | 0.1896 |
| EPCR III | 1277.5 | -1.783 | 0.0746 |
| EMPI | 1261.5 | -1.782 | 0.0748 |
| EMP II | 1214.5 | 1.865 | 0.0622 |
| EMP III | 1650.5 | -0.722 | 0.4701 |
| MMP -2I | 1315.5 | -0.431 | 0.6664 |
| MMP -2 II | 676.5 | 3.801 | 0.0001 |
| MMP -2 III | 1530.5 | 0.380 | 0.7040 |
| MMP -9I | 1313.5 | -0.444 | 0.5730 |
| MMP -9 II | 635.5 | 4.083 | 0.0000 |
| MMP -9 III | 1581.5 | -0.097 | 0.9227 |
| TIMP -2 I | 1262.0 | 0.765 | 0.4440 |
| TIMP -2 II | 1015.0 | 1.470 | 0.1415 |
| TIMP -2 III | 1407.5 | -1.062 | 0.2882 |

sia and controls.

By contrast, no statistically significant differences in the levels of vWF (both antigen and activity), t-PA, EPCR,

DISCUSSION

The group of women with normal pregnancy (controls) Procoagulant factors

During pregnancy, the levels of coagulation factors V, VII, VIII, IX, X, XII, and vWF are significantly elevated. These changes are accompanied by a rise in the level of fibrinogen to as much as twice the values in non pregnant women. In agreement with the previously published data, a significant increase in vWF activity and antigen was shown in our group [17-21].

Anticoagulant factors

The system of natural inhibitors of coagulation plays a key role in maintaining pregnancy. Plasma soluble thrombomodulin may be used as an important marker of endothelial damage. Thrombomodulin levels increase significantly during all three trimesters of pregnancy. Although the level at week 12 cannot be used to predict a potential threat to the pregnancy, a sudden rise in the level might suggest a severe placental complication [22].

Besides thrombomodulin, the protein C system involves protein C, whose levels drop throughout pregnancy, C4b-binding protein and activated protein C. The activity of the system is initiated immediately after thrombomodulin binds to thrombin. The binding is mediated by endothelial protein C receptor, another significant marker of endothelial activation. In agreement with the literature data, an increase in EPCR levels during pregnancy was demonstrated [23].

Fibrinolysis

The activity of the fibrinolytic system is reduced during pregnancy and remains low during labor but rapidly returns to normal immediately thereafter [24]. In our group, the levels of tissue plasminogen activator did not change throughout pregnancy which is consistent with the published data [25]. The levels of t-PA are influenced not only by an increase in PAI-1 levels, as shown in our study, but especially by higher levels of PAI-2 originating from the placental tissue. The level of PAI-1 is several times higher in the 3rd trimester than at the beginning of pregnancy and returns to normal immediately after delivery [26].

Endothelial microparticles

Normal pregnancy is characterized by higher levels of both platelet and endothelial microparticles. Their role in the etiopathogenesis of pathologic pregnancy remains unclear [27]. Vanwijk et al. demonstrated that in women with preeclampsia, but not in healthy pregnant women, microparticles cause endothelial damage in isolated myometrial arteries [16]. In agreement with the previously published data, our group showed a trend of increasing endothelial microparticles during pregnancy [28].

The metalloproteinasesystem

The balance between MMP and TIMP has an important role in vascular remodeling, angiogenesis, and uterine and systemic vasodilation during pregnancy. Impairment in the balance is associated with numerous severe clinical conditions, namely hypertension and preeclampsia during pregnancy. In the 1st trimester, MMP-2 has been shown to be expressed mainly in the extravillous trophoblast and MMP-9 especially in the villous trophoblast. The activity of MMP-2 may be observed until delivery, with cytotrophoblast invasiveness being inhibited by TIMP-2 activity and anti-MMP-2 antibodies. Elevated plasma levels of metalloproteinases were also reported

during normal pregnancy and are thought to be related to vascular changes, mainly in early pregnancy [29]. Our study confirmed a constant increase in the levels of MMP-2, MMP-9, and TIMP-2 without significant alterations throughout pregnancy.

The group of pregnant women with preeclampsia

The pathophysiology of preeclampsia is not completely clear. The condition is assumed to have 2 stages, depending on the course of trophoblast invasion and subsequent endothelial cell dysfunction. The condition is heterogeneous, with a high variability in both clinical manifestations and the onset of problems. Basically, the onset of clinical and laboratory manifestations of preeclampsia may be either early or late. In recent years, the role of oxidative stress in the pathophysiology of preeclampsia has been widely discussed. Placental hypoperfusion followed by reperfusion causes oxidative stress and the resulting products and metabolites damage endothelial cells and activate numerous pathophysiological processes leading to severe changes in the organism of a pregnant woman [27].

Results of studies focused on the mechanism of endothelial damage due to oxidative stress in preeclampsia are often contradictory. This may be related to the different pathophysiology of early- and late-onset preeclampsia.

The results of our study, consistent with the previously published data, show significantly higher levels of von Willebrand factor antigen and activity in the preeclampsia group than in the control group [21]. Von Willebrand factor facilitates platelet adhesion to subendothelial collagen and accelerates formation of the primary hemostatic plug. Similarly to PAI-1 or t-PA, its synthesis is not limited to endothelial cells but vWF is also synthesized by megakaryocytes. However, given the elevation of the other endothelial activation markers, the source of increased vWF levels in preeclamptic women is likely to be the endothelium. So far, it is not known whether endothelial damage is the primary cause or result of these pathological conditions.

In our group, significant differences in thrombomodulin levels in all three trimesters were found in the group of women who developed preeclampsia during their pregnancies, as compared with the control group of women with normal pregnancies. This finding is consistent with the previously published data. Thrombomodulin levels are significantly elevated in pregnant women with preeclampsia and correlate with the severity of the condition [17,21]. Serial thrombomodulin assays may be used to select women who could benefit from early pharmacological intervention [19].

Similar to thrombomodulin, EPCR is a glycoprotein expressed predominantly on the surfaces of vascular endothelial cells, as well as in the placenta. It plays a crucial role in the protein C anticoagulant system. Both markers are characterized by a relatively high genetic variability. EPCR mutations have been described particularly in patients with recurrent pregnancy losses and severe

placental damage - OR 4.0 (95% CI 1.1 - 14.9) [23]. Endothelial damage is associated with elevated plasma levels and concentration of soluble EPCR. In our group, significant elevations were shown in all three trimesters. As compared with the group of healthy pregnant women, the preeclampsia group was characterized by a significant increase in the average levels of EPCR in the 2nd trimester and a non-significant increase in the 1st and 3rd trimesters. This is consistent with the previously published results [17].

The fibrinolytic system depends on a balance between several factors: physiological plasminogen activator inhibitor, urokinase, and tissue plasminogen activator which are the primary regulators of fibrinolysis. In agreement with the published data, no changes in t-PA levels were seen in any of the groups [20]. Besides the endothelium, numerous other cells such as fibroblasts and synovial cells produce t-PA. Similar conclusions were published by Hunt et al. who studied 27 normal pregnancies, 24 pregnancies with abnormal ultrasound findings in the uterine arteries but normal pregnancy outcomes, 24 pregnancies with bilateral abnormalities in the uterine arteries and pathological pregnancy outcomes, and 7 patients with preeclampsia or fetal growth retardation in week 23 [22]. When comparing the markers of fibrinolysis, elevated t-PA levels were found in severe preeclampsia but no differences in the levels between normal pregnancies and the two groups with abnormal ultrasound findings [24].

Plasminogen activator inhibitor-1 is considered to be one of the main markers of endothelial dysfunction in preeclampsia. PAI-2 is produced by placental tissue and is regarded as a marker of placental function. Our study showed significantly higher levels of PAI-1 in the 2nd and 3rd trimesters in the preeclampsia group and significantly elevated levels in women with chronic hypertension (in the 1st and 3rd trimesters, with the 2nd trimester being just below the significance level). No difference was found when comparing the 3rd trimesters in the control group and in women with HELLP syndrome. The results are consistent with the data published earlier. A Swedish study by Wikström et al. yielded significant differences only in women with early-onset preeclampsia but not in those with late-onset disease [25]. Concentration and elevation of PAI-1 in women with preeclampsia are confirmed by some studies but refuted by others [25,26]. This illustrates the complexity of the problem and marked heterogeneity of the condition. The differences in results may stem from the genetically determined polymorphism of PAI, the way of handling the laboratory samples (serum or plasma processing), and also the fact that PAI-1 may be produced, apart from the endothelium, by numerous other cell types such as smooth muscle cells, thrombocytes or neutrophils.

Studies aimed at assessing microparticles in patients with preeclampsia showed lower counts of platelet and higher counts of endothelial microparticles as compared with healthy pregnant controls [17]. The decrease in mi-

croparticle count in these patients is likely to be related with the absolute decrease of platelet count in preeclamptic women. Higher microparticle counts are closely correlated with levels of CRP, a complement activator in this case [27]. However, our study did not reveal a significant increase, with microparticle counts remaining nearly unchanged in women with both normal pregnancies and preeclampsia. We decided to use flow cytometry based on anti-CD144 immunotyping [28]. The nonsignificant increase might be due to the use of a different antigen than in other works [29]. Despite numerous studies, the role of microparticles in the pathogenesis of preeclampsia remains unclear. One reason may be the activation of tissue factor expressed on the surface of monocytes. The complexity of the problem is illustrated by a study by Vanwijk et al. They studied bioptic samples collected from the uterus of 22 women during cesarean section to ascertain whether plasma of preeclamptic women may cause endothelial dysfunction. After incubation of samples with whole plasma, microparticle-free plasma, and isolated microparticles resuspended in saline solution, bradykinin concentration was measured. Endothelial dysfunction was not observed if plasma from women with preeclampsia was used, either with or without microparticles. Statistically significant effects were only seen in the case of isolated microparticles obtained from preeclamptic women [16]. The complex metalloproteinase system in patients with preeclampsia and hypertensive disorders was studied by many authors. Poon et al. reported a significant predictive relationship in MMP-2 and MMP-9 in patients with late-onset preeclampsia [7]. Palei et al. studied MMP-2, MMP-9, and TIMP-2 in the course of all three trimesters, with a significant elevation in MMP-9 but not MMP-2 [29]. Similar results were seen in our group - a significant increase in MMP-9 in the 2nd trimester and a nonsignificant increase in the 3rd trimester.

CONCLUSION

We confirmed the hypothesis regarding the significant influence pregnancy and preeclampsia has on changes in levels of these markers.

Clinical Message:

Placental ischemia with endothelial dysfunction and generalized flushing of circulating factors into the maternal circulation was involved in the development of preeclampsia. The aim of the study was to find a laboratory marker for detecting endothelial activation during physiological pregnancy by the determination of specific markers and comparing the values with the values found in women who developed preeclampsia. The most important finding was the possible predictive potential of thrombomodulin and PAI-1.

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Declaration of Interest:

The authors declare there are no conflicts of interest.

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