Expression Of Urokinase Plasminogen Activator, Its Receptor And Plasminogen Activator Inhibitor In The Plasma Of Pathological Pregnancy Women

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Citation

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Abstract

Objective: The aim of the work was to study the fibrinolytic system during pregnancy and after delivery and establish a reference for analogical data in pathological pregnancies. The urokinase-type plasminogen activator (uPA), its receptor (uPAR) and plasminogen activator inhibitor-1 (PAI-1) are serum proteases involved in the fibrinolytic system. We measured them in healthy pregnant women in the first, second and third trimester of pregnancy, at the time of delivery, and after delivery. The plasma from 157 women was analysed for expression of the uPA, PAI-1 and uPAR using enzyme-linked immunosorbent assay (ELISA).

Results: We observed that the values of PAI-1 and uPA increased systematically during pregnancy. They were the highest in the third trimester and the lowest after delivery (P≤ 0.0001). On the other hand, the value of uPAR decreased during pregnancy.

Conclusion: The levels of uPA and PAI-1 increased gradually during pregnancy and decreased sharply between the first stage of labour and 24-48 hours after delivery; when they reached the level lower than in the first trimester. The level of uPAR decreased during three trimesters.

INTRODUCTION

The invasion of trophoblast, accompanied by degradation of extracellular matrix, is crucial to normal pregnancy development. The fibrinolytic system plays a key role during pregnancy, labour and puerperium. Pathological placental invasion and pathological implantation probably plays a role in many obstetrical diseases, such as preeclampsia [1]. The development of the hemochorial placenta requires the trophoblast to invade the endometrium, penetrating into blood vessels. Placental tissue includes components from two haplodifferent organisms. Early steps in trophoblastic development are the adhesion and invasion, which is an active process. There are correlations observed between invasion and proteinase production, like uPA, tPA and PAI-1 [2]. Chou et al. [3] suggest that gonadotropin-releasing hormones differentially regulate the balance between uPA and PAI-1 expression levels in the human decidua, possibly via distinct receptor-mediated pathways. Koh et al. [4] study suggests that at hydrops fetalis, further enhanced prothrombin formation and hyperfibrinolysis/inhibitor at late mid-trimester is associated with a poor obstetric outcome. The plasminogen activation system and matrix metalloproteinases (MMPs) play a key role in the degradation of basement membrane and extracellular matrix in tissue remodeling, cancer cell invasion and metastasis. uPA and PAI-1 are involved in breast cancer development, and increased mRNA expression may be associated with a worse prognosis [5]. The study from Teesalu et al. [6] shows that in murine hemochorial placentation uPA has an essential role in the maintenance of the fibrinogenic/fibrinolytic balance in the decidua.

The human placenta is a highly invasive structure in which a subpopulation of placental trophoblast cells invades the decidua and its vasculature to establish adequate fetal-maternal exchange. It was found that molecular mechanisms responsible for this invasion are identical to those of cancer cells; however, “unlike cancer cells, their proliferation, migration, and invasiveness in situ are stringently controlled by decidua-derived transforming growth factor (TGF)-beta” [7]. Liu et al. [8] demonstrate that “uPA: uPAR interaction

stimulates extravillous trophoblast cell migration, independent of uPA enzymatic activity, using the mitogen-activated protein kinase pathway and calcium signaling events including the participation of PI 3-kinase and phospholipase C." These findings may be important for such clinical conditions as spontaneous abortion, preeclampsia, and choriocarcinoma.

uPA binds to its receptor, uPAR, on the surface of cancer cells, leading to the formation of plasmin. Rhabdomyosarcoma (RMS) cell lines secrete high levels of insulin-like growth factor II (IGF-II), which may indicate that autocrine IGFs play a major role in the unregulated growth and metastasis of RMS [9]. Choi and Pai [10] show that tPA levels change in parallel with plasma fibrinogen concentrations during and after normal pregnancy. uPA and uPAR are central molecules for uPA/uPAR/plasmin-dependent proteolysis, which is thought to play a significant role in the development of pregnancy as well as in its many complications: pre-term pre-mature rupture of foetal membranes and placental abruption [11]. Aflalo et al. [12] indicate that in vitro embryo development leads to lower PA and supports the role of uPA in the implantation process. The rise in endometrial uPA may also indicate its importance for this process. In our work, we present the results of uPA, uPAR and PAI-1 activity in the plasma of pregnant women at different stages of healthy pregnancy as compared with the results of plasma values in women during and after delivery. We believe that organizing these data and finding a pattern in the change of uPA, PAI-1 and uPAR levels will be relevant for further study of pregnancy pathology.

MATERIALS AND METHODS

PREGNANT WOMEN

The plasma was collected from pregnant women in the 1st Clinic of Obstetrics and Gynecology, Wroclaw Medical University. In our study we analyzed the expression of uPA, PAI-1 and uPAR in plasma of healthy pregnant women. We divided them into three groups: the first, the second, and the third trimester of pregnancy, 31 women in each. The fourth group (31 women) was made up of women during the first stage of labour and in the fifth group there were 33 women at 24-48 hours after delivery. All cases with processes involving fibrinolytic activation were excluded. The samples of plasma were frozen at -80°C.

DETERMINATION OF UPA, PAI-1 AND RECEPTOR (UPAR)

Blood sampling and the preparation of plasma for determining PAI-1, uPA and uPAR were made according to Kluft and Meijer's recommendations [13]. Quantitative measurements of PAI-1, uPA and uPAR were carried out utilizing ELISA kits. The required dilutions, antibodies, conditions, and detection ranges for each ELISA were prepared according to the manufacturer instructions. The concentration of each protein was measured at 450 nm on a microplate reader (Dynex Technologies, Billinghurst, UK). The values for PAI-1, uPA and uPAR (ng/ml) were determined for each sample from a standard curve using Revelation Software (Dynex Technologies, Billinghurst, UK).

STATISTICAL ANALYSIS

The data were expressed as the mean values ± SD. Walloon's rank test were used. The 0.05 level of probability was assumed as significant. The significance of the differences in median values of uPA, PAI-1 and uPAR wes calculated by Wilcoxon matched pairs signed-rank test.

RESULTS

The values of uPA in the first trimester (median 0.9 ng/ml, range 0.6–2.8) were statistically significantly higher ($p \leq 0.0005$) than after delivery (median 0.6 ng/ml, range 0.2–1.3). The values of PAI-1 in the first trimester (median 141.0 ng/ml, range 33.2–242.3) were also statistically higher than after delivery (median 77.8 ng/ml, range 20.8–196.0) ($P \leq 0.0001$). The values of uPAR in the first trimester were higher (median 1.5 ng/ml, range 0.7–2.9) in comparison with the women after delivery (median 0.9 g/ml, range 0.5–2.0) ($P \leq 0.0005$). PAI-1 and uPA values increased also significantly in the second and third trimester in comparison with the first trimester and after delivery ($P \leq 0.0001$), while uPAR values decreased during pregnancy, but this was not statistically significant. The values of uPA in the second trimester were: median 1.5 ng/ml, range 0.6–2.2. The values of PAI-1 were: median 207.7 ng/ml, range 52,5–540.0. The values of uPAR in the second trimester were: median 1.4 ng/ml, range 0.6–3.4. In the third trimester and during labour the values of uPA were: median 2.1ng/ml, range 0.9–6.1 and median 2.0 ng/ml, range 0.5–3.4, respectively. PAI-1 values in the same groups were: median 268.8 ng/ml, range 120.2–1073.0 and median 210.5 ng/ml, range 77.4–699.5, respectively. The values of uPAR decreased in women during labour, compared with those in the third trimester.

The values were: median 1.2 g/ml, range 0.5–2.6 and median 1.3 g/ml, range 0.7–3.0, respectively. The results for uPA, PAI-1, and uPAR are presented in (Fig 1). It was found that uPA and PAI-1 increased statistically significantly in all trimesters of pregnancy in comaparison with women after delivery (P ≤0.0001). These values incresed significantly in the third trimester as compared with the first and the second trimester and during labour (P ≤0.0001). The mean value of uPA in the plasma of women in the first trimester of pregnancy was 1.1±0.5, compared to 2.1±0.9 ng/ml in the third trimester and to 0.7.±0.3 ng/ml after delivery (Fig 1A). The mean value of PAI-1 in the first trimester was 221.7±106.5 ng/ml, compared to 333.2.±208.2 ng/ml in the third trimester and 82.1±50.0 after delivery (Fig 1B). No statistically significant changes were observed in mean uPAR value in the first trimester to the time of delivery (Fig 1 C). The results from (Table 2) shows that the hazard ratios represented the difference between the pathological stages in all teimester of pegnency compared with women after delivery, the uPA and PAI-1 ratios increased from 1.5 and 1.8 in first trimester to 3.5 respectively. While uPAR decreased from 1.7 in first trimester to 1.4 (1.2-fold). uPA and PAI-1 were increased 2.3-fold in comparison with women after delivery (P ≤0.0001).

Figure 1

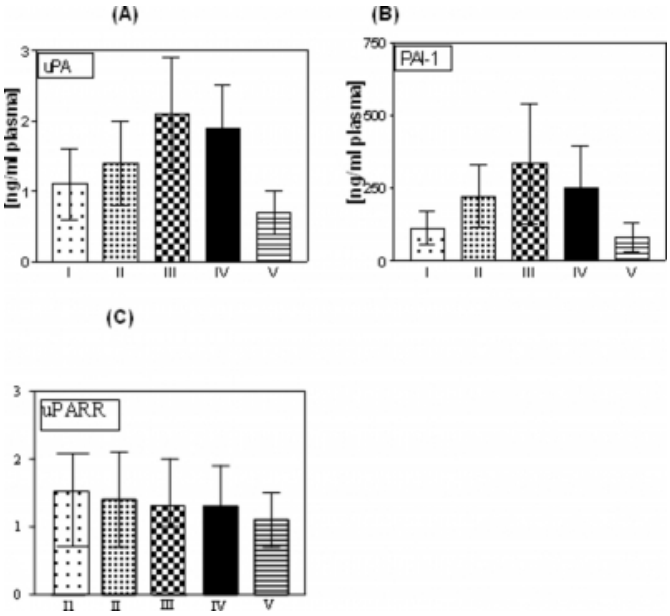
Table 1: Median value of uPA, PaI-1 and uPAR. The values are in ng/ml of plasma

Group	uPA Median Range [25-75 percentile] 95% CI*	PAI-1 Median Range [25-75 percentile] 95% CI*	UPAR Median Range [25-75 percentile] 95% CI*
Trimester I	0.9 0.6-2.8 0.8-1.1 0.9 to 1.4	141 33.2-242.3 72.4-141 89.4 to 136.4	1.5 0.7-2.9 0.9-1.4 1.1 to 1.6
Trimester II	1.5 0.6-2.2 0.9-1.9 1.1 to 1.7	207.7 52.5-540.0 138.1-251.3 161.8 to 261.5	1.4 0.6-3.4 0.9-1.5 0.9 to 1.3
Trimester III	2.1 0.9-6.1 1.7-2.2 1.8 to 2.4	268.8 120.2-1073.0 188.7-412.3 259.4 to 407.1	1.3 0.7-3.0 1.1-1.6 1.3 to 1.6
First stage of labour	2.0 0.5-3.4 1.5-2.1 1.6 to 2.1	210.5 77.4-699.5 150.0-310.6 203.2 to 303.4	1.2 0.5-2.6 0.9-1.3 1.1 to 1.5
24-48 h after delivery	0.6 0.2-1.3 0.5-0.8 0.6 to 0.8	77.8 20.8-196.0 49.5-89.0 61.5 to 102.7	0.9 0.5-2.0 0.9-1.3 0.8 to 1.3

Median value of uPA, PaI-1 and uPAR. The values are in ng/ml of plasma

Figure 2

Figure 1: Values in ng/ml (mean ±SD) of uPA, PAI-1 and uPAR.



I) The first trimester
II) The second trimester
III) The third trimester
IV) The first stage of labour
V) 24-48 hours after delivery

Figure 3

Table 2: Statistical analysis for pathological stages of pregnancy women

Pregnancy trimester	uPA 95% CI[a]	HR[b]	PAI-1 95% CI[a]	HR[b]	UPAR 95% CI[a]	HR[b]	Significance *P*
Trimester I (31 women)	0.9 to 1.4	1.5	89.4 to 136.4	1.8	1.1 to 1.6	1.7	0.0001
Trimester II (31 women)	1.1 to 1.7	2.4	161.8 to 261.5	2.7	0.9 to 1.3	1.6	0.0001
Trimester III (31 women)	1.8 to 2.4	3.5	259.4 to 407.1	3.5	1.3 to 1.6	1.4	0.0001
Delivery time (31 women)	1.6 to 2.1	3.3	203.2 to 303.4	2.7	1.1 to 1.5	1.3	0.0001

The significance of the differences in median values of uPA, PAI-1 and uPAR were calculated by Wilcoxon matched pairs signed-rank test
CI[a] = confidence interval
HR[b]= hazard ratio between the pathological stages of women pregnancy and with women after delivery

DISCUSSION

The suppression of fibrinolytic activity plays an important role in the prevention of hemorrhage during pregnancy and labor. A hypofibrinolytic and hypercoagulable state may be established in the placenta during pregnancy [10,14]. Guan et al. [15] show that stromal cells from endometrium and endometriotic tissues release uPA, soluble uPAR and PAI-1. The release is partly hormonally regulated, but differently in endometriotic than in endometrial cells. In our study we measured uPA, uPAR and PAI-1 in plasma of pregnant

women in their first, second, third trimester of pregnancy, during labour and 24-48 hours after delivery. We observed that PAI-1 and uPA values increased significantly in the first, second and third trimester in comparison with women after delivery ($P \leq 0.0001$), while the uPAR values decreased, although not statistically significantly. The antigen levels of PAI-1, PAI-2 and uPA increased linearly during pregnancy and promptly returned to the level on non-pregnant women after delivery [16]. The plasminogen/plasmin system, uPA, uPAR, and PAI-1, influences extracellular proteolysis and cell migration in lung injury or neoplasia. The induction of PAI-1 by exposure of lung epithelial cells to uPA is a newly recognized pathway by which PAI-1 could regulate local fibrinolysis and urokinase-dependent cellular responses in the setting of lung inflammation or neoplasia [17]. A significant increase in PAI-1 antigen and plasminogen activator inhibitor activity was observed in preeclampsia, with or without intrauterine growth retardation, but not in normotensive pregnancy with intrauterine growth retardation (IUGR). Plasminogen activator inhibitor type 2 (PAI-2) antigen levels showed a significant decrease in both groups of pregnant women (normotensive or preeclamptic) with IUGR and without IUGR. A significant correlation between PAI-2 levels and fetal weight has been observed in the clinical groups [18]. In our groups there were no pregnancies with IUGR. This will be the subject of further study. The results from (Table 2) shows that the hazard ratios represented the difference between the pathological stages in all teimester of pegnency compared with women after delivery, the uPA and PAI-1 ratios increased from 1.5 and 1.8 in first trimester to 3.5 respectively. While uPAR decreased from 1.7 in first trimester to 1.4 (1.2-fold). uPA and PAI-1 were increased 2.3-fold in comparison with women after delivery ($P \leq 0.0001$). The predominance of PAIs over tPA is maintained throughout the whole labor, though at lower levels, as can be inferred from the tPA/PAIs ratio [11, 19].

It was observed that proteolitytic enzymes, especially metalloproteinase activators of plasminogen or cysteine end peptidases, play a very important role in the interaction between the developing placenta and the decidua [20]. From the information obtained so far it becomes clear that the development of placenta is accompanied by an increase in specific proteolytic enzymes whose activities are controlled by specific inhibitors. It was noticed that these processes are controlled by specific proteins produced in the placenta [21,22]. In conclusion, we observed that the activities of uPA and PAI-1 increased gradually during pregnancy and decreased dramatically between the first stage of labour and 24-48 hours after delivery, when they reached the level lower than in the first trimester.

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