# Regulation The Expression Of Plasminogen Activator And Interluekin-6 In Women Plasma By Vitamin E After Abdominal Gynecological Operation

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**Citation**

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**Abstract**

Objective: The purpose of this study was to determine whether there is a stimulation of production of uPA, PAI-1 and interleukin-6 before and after operation in plasma patients with gynecological diseases, and whether vitamin E could inhibit this process.
Methods: uPA, PAI-1 and interleukin-6 were determined by enzyme linked immunosorbent assay (ELISA) in non oncological diseases (n=34) and in healthy individuals (n=20) and correlated with vitamin E treatment after operations.
Results: Our results show that uPA, PAI 1, and interleukin-6 values were significantly higher in non oncological gynecological cases in comparison with healthy individuals (8.3 times, 3.8 times, and 9.8 times, respectively, P≤ 0.0001). uPA, PAI-1, and interleukin-6 were significantly higher after abdominal gyneocological operation than before operation in the group of patients who were not treated with vitamin E after operation (P≤ 0.0001). uPA and interleukin-6 decreased significantly after abdominal gyneocological operation in the patients treated with vitamin E in comparison with the group of patients without such treatment (P≤ 0.0001).
Conclusion: These findings contribute to the better understanding of a potential cellular mechanism for the beneficial effects of antioxidant therapy in gyneocological disease patients.

## INTRODUCTION

During the whole period of woman life there are many processes which involve degradation of extracellular matrix (tissue morphogenesis, angiogenesis, bone remodelling, wound healing, trophoblast implantation, involution of postpartum uterus or involution of postlactation mammary gland). Also, proteolysis is found in pathological conditions such as tumor growth, metastasis, arthritis and autoimmune diseases ([1]). Urokinase plasminogen activator (uPA) and urokinase plasminogen activator receptor (uPAR) are the central molecules for uPA/uPAR/plasmin-dependent proteolysis, which is thought to play a significant role in the development of pregnancy, as well as its many complications during pregnancy. The results support the hypothesis that the fibrinolytic system participates in preterm rupture of membranes and placental abruption ([2]). Aflalo et al., ([3]) indicate the increased activity of uPA in the blastocyst stage and in the implantation. Moreover, the rise of endometrial uPA indicates its role in this process. Bruse et al. ([4]) found that uPA mRNA seems to be up-regulated in endometriotic glands and endometrial stroma as well as PAI-1 mRNA in endometriotic and endometrial stroma from women with endometriosis.

High intake of n-6 fatty acids may attenuate beneficial effects of n-3 fatty acids. These results suggest that the inhibition of inflammatory cytokines may be one possible mechanism for the observed beneficial effects of fatty acids on chronic inflammatory diseases ([5]). Son et al., ([6]) suggest that low-density lipoprotein (LDL) regulates PAI-1, uPA, and tPA in biphasic patterns in HMC, and the up regulation of PAI-1, uPA, and tPA after long-term LDL exposure seems to be mediated by a delayed protein kinase C (PKC) activation associated with an increased PA inhibitory activity. These results suggest that LDL, after prolonged incubations with HMC, causes a PA/inhibitor imbalance favoring accumulation of matrix. The plasminogen activator (PA)-plasmin proteolytic system has recently received considerable attention because of its participation in a wide

variety of biological activities and in pathological conditions involving tissue destruction. These results suggest that IL-6 stimulated PA activity through an enhancement of tPA gene expression and may be involved in extracellular matrix degradation through the stimulation of the PA-plasmin system of human dental pulp (HDP) cells ([7]). The inflammatory cytokine interleukin-6 (IL-6) is a powerful inducer of the hepatic acute-phase response, and it has been proposed to be a central mediator in the pathogenesis of coronary heart disease through a combination of autocrine, paracrine, and endocrine mechanisms ([8]). In fact, in a recent study, serum levels of IL-6 were predictive of the risk of myocardial infarction in apparently healthy individuals, and although the levels of IL-6 were strongly correlated with the levels of C-reactive protein, the association between IL-6 and the risk of myocardial infarction remained significant, even after adjustment for the C-reactive protein level ([9]). We investigated the effects of vitamin E on urokinase-type PA (uPA), in relation to the plasma interleukin-6 production in women after abdominal operation because of non oncological gynecological disease. We tried to show that vitamin E could inhibit this process.

## MATERIALS AND METHODS

Venous blood samples from the study (34 women undergoing abdominal gynecological operation: amputatio uteri, exstirpatio uteri or ovariectomia) and the control group (20 healthy women in the same age) were collected from the antecubical vein. The control group consisted of 20 healthy individuals who were invited for routine gynecological examination and Pap smear (complimentary). The study group was further divided into 2 subgroups: 17 women undergoing gynecologial operation for non oncological diseases (mainly myomata uteri - 16, and benign ovarian tumors - 1) and not treated with vitamin E after the operation and another 17 women undergoing gynecologial operation for non oncological diseases (mainly myomata uteri - 15 and benign ovarian tumors - 2) treated orally with vitamin E after the operation. Mean age in all groups was 48 years. All cases with processes involving fibrinolytic activation (described in literature) were excluded. Blood was collected in plastic tubes with 3.8 % sodium citrate about 9 a.m. The collected blood was mixed immediately by inverting the tube, cooled with ice-cold water and centrifuged at 2000 xg for 10 minutes to remove the plasma. The plasma samples were stored frozen (–80 °C) until used. 17 operated patients were treated orally with 800 mg vitamin E daily for 7 days (vitamin E was obtained from Hasco, Wroclaw, Poland). Another 17 operated patients were not treated with vitamin E. The blood from operated women was collected for the first time the day before operation, for the second time on the first day after the operation and for the third time on the $7^{th}$ day after the operation.

The concentrations of uPA, PAI-1 and interleukin-6 were measured by enzyme immunoassay kits. The required dilutions, antibodies, conditions, and detection ranges for each ELISA were done according to protocol procedures ([11]). Serum level of IL-6 was measured with commercially available quantitative “sandwich” enzyme-linked immunosorbent assay (Quantikine) kits obtained from R&D System using specific antibodies. The concentration of each protein was measured at 450 nm on a microplate reader (Dynex Technologies, Billinghurst, UK). Values for the PAS components (ng/ml) and interleukin-6 (pg/ml) were determined for each sample from a standard curve using Revelation Software (Dynex Technologies, Billinghurst, UK). Final plasma values were expressed as ng/ml or pg/mg protein for PAS components and interleukin-6, respectively.

The amount of vitamin E was determined by liquid chromatography, using a HPLC apparatus (“Philips”) and a Pye Unicam PU 4020 UV detector. The results were decoded with use of Peak Simple Chromatography Data System Program ([11]).

Values are expressed as mean ± SD. The significance of the mean differences between groups was assessed by the Student's two-tailed unpaired t test. The correlation coefficient was determined by linear regression analysis. Differences were considered significant at $p<0.05$.

## RESULTS

In our study, paired non oncological cases before operation compaired with haelthy individuals were analysed for expression of interleukin-6, uPA, and PAI-1. uPA values were significantly higher in non oncological gynecological diseases (median 2.5 ng/ml, range 0.6–7.3) than in healthy individuals (median 0.3 ng/ml, range 0–3.6; $P\leq 0.0001$). PAI-1 values were significantly higher in non oncological cases (median 10.6 ng/ml, range 5.2–20.4) than in healthy individuals (median 2.6 ng/ml, range 0.5–5.7; $P\leq 0.0001$). Interleukin-6 values were significantly higher in non oncological cases (median 42.4 ng/ml, range 20.5–616) than in healthy women (median 4.3 g/ml, range 1.7–8.4; $P\leq 0.0001$) ( Fig 1. A, B). uPA, PAI-1, and interleukin-6 increased significantly on the first day after abdominal

gynecological operation in comparison with these values before operation in the same women (P≤ 0.0001) (Fig 1C). uPA values were: median 0.94 ng/ml, range 0.55-1.8 (P≤ 0.0001). PAI-1 values were: median 167.3 ng/ml, range 78.5-262.6 (P≤ 0.0001).

Figure 1: Changes in uPA, PAI-1, and interleukin-6 concentrations in women plasma with non-oenological cases in comparison with control (healthy).

**Figure 1**

Figure 1a: Healthy individuals women (control).

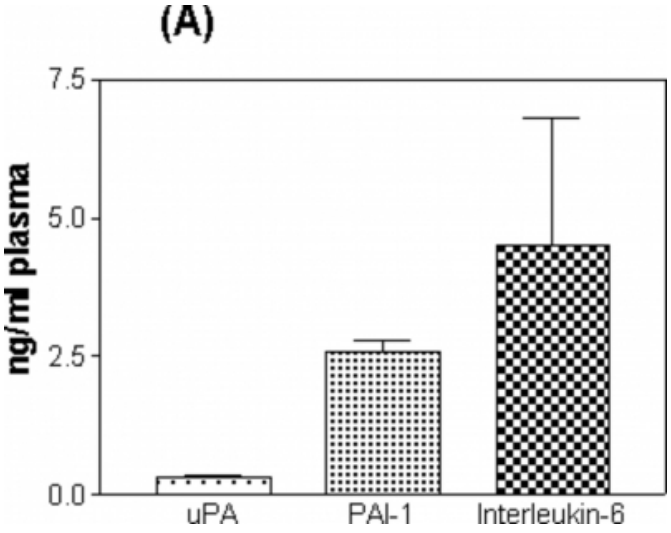



**Figure 2**

Figure 1b: Patients with non-oenological cases

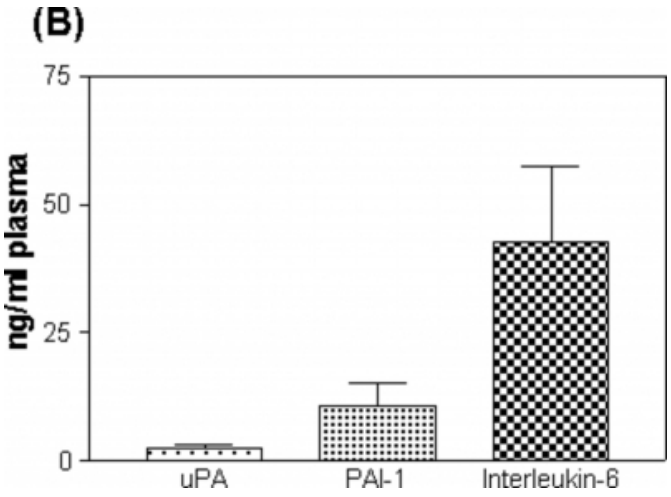
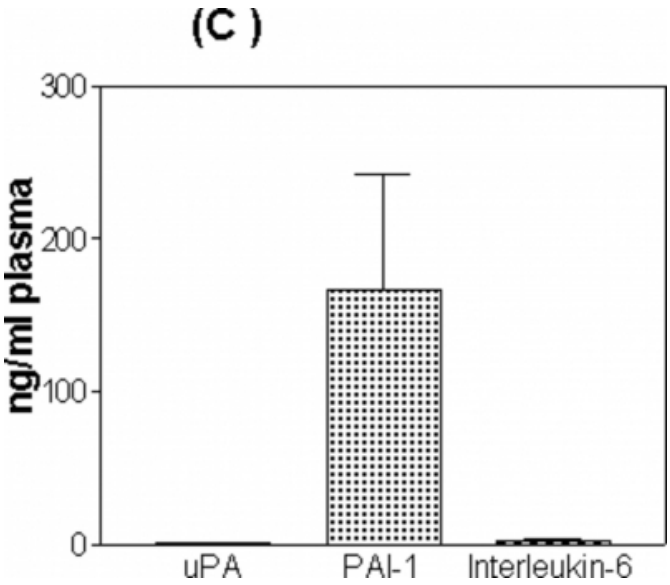



**Figure 3**

Figure 1c: Patients after operation




The significance of the differences in median values of patients with non-oncologecal cases and Healthy individuals women (control) were calculated by Wilcoxon matched pairs signed-rank test. Differences were considered significant at $p<0.05$.

Interleukin-6 values were: median 2.5 ng/ml, range 1.2-4.6 (P≤ 0.0001). After abdominal gynecological operation the patients treated daily with 800 mg vitamin E for 7 days had the uPA and interleukin-6 levels significantly lower than patients without such treatment (P≤ 0.0001). The level of PAI-1 was not measured in the group with vitamin E treatment (there was no funding for that in our grant). uPA values were: median 0.5 ng/ml, range 0.1-1.6 (P≤ 0.0001). Interleukin-6 values were: median 2.6 ng/ml, range 0.7-3.4 (P≤ 0.0001) after gynecological operation and treatment with vitamin E (Fig. 2B). Obviously, the concentration of vitamin E increased in women treated with vitamin E in comparison with women not treated so (P≤ 0.0001). Vitamin E values were 2.6± 1.0 ng/ml before treatment and 8.6± 3.2.0 ng/ml after treatment. uPA, PAI-1, and interleukin-6 values within after abdominal gyneocological operation the pateints treated daily with 800 mg vitamin E correlated significantly with each other ($P < 0.0001$; $r=0.925$). In addition, interluekin-6 expression correlated with PAI-1 expression ($P < 0.0001$; $r=0.995$).

Figure 2: Changes in uPA, interleukin-6 and vitamin E concentrations in women plasma with non-oencological cases

**Figure 4**

Figure 2a: Patients after operation and without treatment with vitamin E

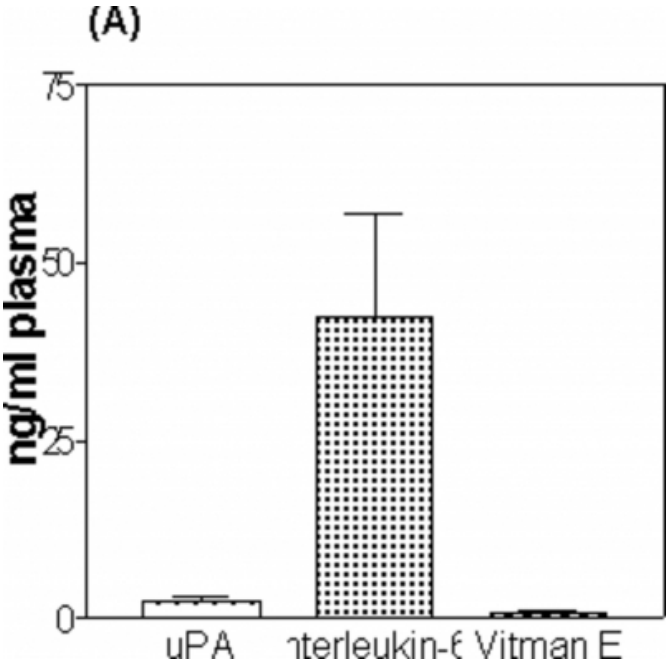
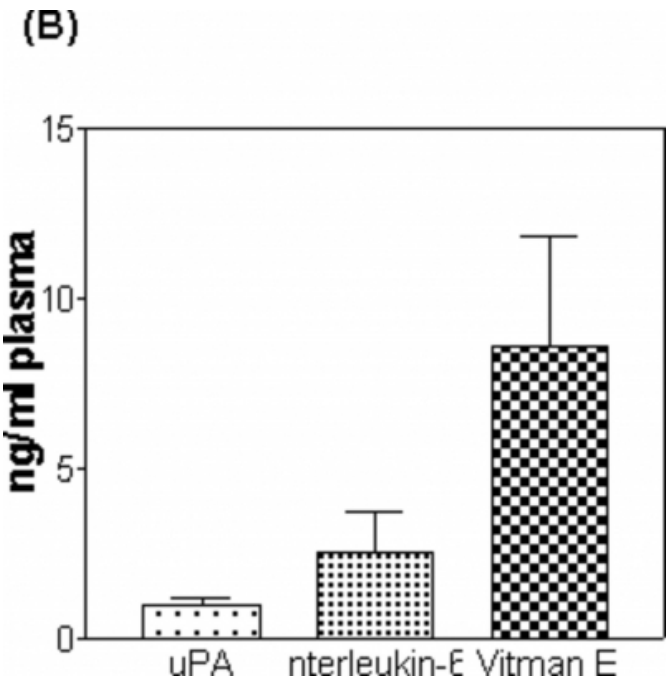



**Figure 5**

Figure 2b: Patients after operation and after treated with vitamin E




The significance of the differences in median values of patients after operation and without treatment with vitamin E and patients after operation and after treated with vitamin E were calculated by Wilcoxon matched pairs signed-rank test. The correlation coefficient was determined by linear regression analysis. Differences were considered significant at $p<0.05$.

## DISCUSSION

The female reproductive system seems to be protected from infection, destruction and oncogenesis. The objective of this study was to determine if there is stimulation of production of uPA, PAI-1 and interleukin-6 in women with non oncological gynecological diseases before and after operation. We also wanted to know whether vitamin E could inhibit this process. The different levels of uPA, PAI-1 and interleukin-6 in non oncological gynecological diseases and in healthy individuals confirm that the reproduction system is a rich source of these markers, and the levels of these markers change not only in oncological and inflammatory diseases. These levels are also changed in cases of myomata uteri and benign ovarian tumors. uPA is involved in serum degradation and PAI-1 in fibrinolysis ([2]). The balance between the expression of the activators and inhibitors is important in vivo in determining whether matrix degradation and activation of other proteinases is likely to occur. In our study, both uPA and the inhibitor PAI-1 were greater in women with non oncological diseases than in healthy individuals. However, previous studies showed that this increase of PAI-1 does not significantly inhibit uPA activity in primary tumours; therefore, the balance will favour proteolysis ([13]), which can also take place in our cases. Our results show that uPA, PAI-1, and interleukin-6 levels were significantly higher in non oncological gynecological cases in comparison with healthy individuals ($P\leq 0.0001$). The increase was: 8.3 times, 3.8 times, and 9.8 times, respectively. Also uPA, PAI-1, and interleukin-6 increased on the first day after abdominal gyneocological operation in comparison with the values before operation ($P\leq 0.0001$) (Fig 1C). Pawelec at al ([14]) in an earlier study demonstrated that the highest concentration of uPA and PAI-1 is within 24 hours and on the 7$^{th}$ day after the operation. That could mean that the fluctuations in the level of uPA and PAI-1 indicate that the healing is a two-stage, or even multi-stage process. They also demonstrated that the highest concentration above the normal value was observed in PAI-1, as compared with uPa and uPAR. These results suggest that PAI-1 more than anything else could be a marker of healing. Wang et al. ([15]) observed that at least one means by which relaxin promotes pig uterine growth is by increasing uterine secretion of uPA. In addition, these studies suggest that relaxin administration in vivo to prepubertal girls has tissue-specific effects with

respect to plasminogen activator. tPA seems to be involved in ovulation, and blockage of ovulation and subsequent cyst formation results from inadequate tPA activity in manipulated follicles ([16]). The plasminogen activator-plasmin system seems to deserve the greatest attention among the active proteolytic cascades of the cell environment. Initially, this system was considered the main system responsible for fibrinolysis. However, the increasing amount of data on a wide spectrum of physiological and pathological conditions associated with the expression of this system contributed to development of the concept on its involvement in the regulation of turnover of a wide spectrum of extracellular matrix components ([16], [17]).

The cascade of proteolytic reactions triggered by the plasminogen activators is started by the conversion of the inactive proenzyme plasminogen into the active enzyme plasmin, a protease of wide specificity, which can directly cleave fibrin/fibrinogen, blood coagulation factors V/Va and VIII/VIIIa, some growth factors, and extracellular matrix components, and can also catalyze the degradation of plasmin-resistant matrix proteins, such as natural collagens, due to activation of inactive zymogens of collagenases (matrix metalloproteinases); thus, it can play the dominant role in extracellular proteolysis throughout the body ([18],[19], [20]). The dominant role of plasmin was confirmed only within the last 15 years, when plasminogen synthesis and plasmin activation were reported to occur everywhere in the body ([21]). During the development process of neoplasmatic disease, the activity of these enzymes increases, they activate other proteolytic enzymes like elastase, kolagenase which causes catalytic degradation of healthy tissues by so doing initiate the development of a disease and their autogenic inhibitors are not able to stop the developing changes ([22], [23]). After abdominal gyneocological operation the patients treated daily with 800 mg vitamin E for 7 days had uPA and Interleukin-6 levels decreased in comparison with the group of patients without such treatment ($P \leq 0.0001$). It is probable that vitamin E induces an increase in the level of kininogen (an autogenic inhibitor of cysteine peptidase), which plays a key role in the transformation of neoplasmatic cells, invasion and metastases and it also controls the formation of apoptotic and necrotic changes ([24]). The data from Baer et al. ([25]) provide evidence that diet can modulate markers of inflammation. Although stearic acid minimally affects LDL cholesterol, it does appear to increase fibrinogen concentrations. In addition, future follow up of the patients involved is required to determine the prognostic relevance of these factors.

## CORRESPONDENCE TO

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