

# The selectins E and P in normal labour. A preliminary report on evaluation of their prognostic values for preeclampsia

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## Abstract

**Purpose:** Our question was whether two biochemical markers of preeclampsia, E-selectin and P-selectin, keep their prognostic value in particular stages of labour, or they lose it during labour.

**Material and methods:** The study group consisted of 36 healthy parturient women who gave physiological births (in control 15 healthy, age-matched women at term). Blood samples were collected in the 1st, 2nd and 3rd stages of labour as well as two hours after placenta expulsion. The levels of soluble E- and P-selectins were measured by immunoenzymatic method (ELISA).

**Results:** The levels of soluble E- and P-selectins in blood plasma of labouring women were within the similar medians and ranges during the 1st, 2nd and 3rd stages of labour as well as two hours after labour ( $p > 0.05$ ).

**Conclusion:** The natural labour does not influence the level of soluble E-selectin and soluble P-selectin in blood plasma of labouring women, therefore the prognostic values of these markers of preeclampsia are preserved in the time of labour.

**Key words:** E-selectin, P-selectin, biomarkers of preeclampsia, labour.

## Introduction

Preeclampsia is still one of the leading causes of maternal deaths (haemorrhages, sepsis and preeclampsia) and contributes also negatively to foetal development (intrauterine growth retardation) [1,2]. The markers of preeclampsia facilitate the supervision of preeclamptic women. The following biomarkers are recommended: plasma soluble E-selectin [3] and plasma soluble P-selectin [4], as well as some other adhesive molecules like VCAM-1 (vascular cell adhesion molecule-1), ICAM-1 (intercellular adhesion molecule-1) [5-8] and vWF (von Willebrand factor) [9], and such substances as thrombomodulin, fibronectin and some others [10,11].

E- and P-selectin as well as L-selectin are members of selectin family and members of a large group of adhesion molecules. They all exist in soluble forms in blood plasma, though each is synthesized or stored in different cells: E-selectin in endothelial cells, P-selectin in alpha granules of blood platelets and Weibel-Pallade bodies of endothelial cells, and L-selectin in leukocytes. Under activating conditions (inflammatory and thrombogenic challenges, as well as preeclampsia), the selectins are expressed on cell membrane and thus can initiate interactions between endothelial cells, leukocytes and platelets (tethering and rolling of leukocytes on endothelial cells, adhesion of platelets to leukocytes, weak and firm adhesion followed by extravasal migration of leukocytes).

The selectins show structural similarities and some overlapping functions [12]. In preeclampsia, the elevated level of E-selectin reflects dysfunction of endothelial cells, while P-selectin represents activation of platelets that can be engaged in the transformation of preeclampsia to HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome [13]. L-selectin plays a major role in early embryogenesis [14].

The molecular activity of P-selectin [15-17] has been described in a particularly precise way. Once P-selectin is expressed on cell membrane, it can bind to its ligand – PSGL-1 (P-selectin ligand-1) which at the same time is

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expressed on activated endothelial cells and leukocytes. The role of selectins in pathogenesis of preeclampsia is at present still a subject of research and discussion. It is likely that the selectins co-operate with other adhesive molecules including integrins, cadherins, immunoglobulin superfamily members, von Willebrandt factor (vWF) and fibronectin, resulting in necrotic inflammation of vascular wall and disseminated intravascular coagulation (DIC) [18].

Predictive and prognostic values of E- and P-selectins as preeclamptic markers have been studied during pregnancy, but not yet in the course of labour. This is exactly why it is not certain whether the level of these markers do not change during labour. If that were the case then they would lose their predictive value at labor time.

In our hypothesis we assumed that the levels of E- and P-selectins in blood plasma can change (increase or decrease) under the influence of delivery itself.

## Material and methods

### Patients

The study group consisted of 36 parturient women at term (gestational age:  $38.8 \pm 0.6$  weeks) of age  $24.3 \pm 2.6$  years, 20 primiparous and 16 nulliparous with the normal course of pregnancy and labour (we excluded from analysis complicated pregnancy, such as preeclampsia, pregnancy induced hypertension, placenta previa, low lying placenta, prolonged rupture of fetal membranes and intraamniotic infection). They were admitted to the hospital at the beginning of labour (1A stage).

Fifteen healthy women at term, age- and parity-matched with the study group, awaiting on labour in hospital, served as control.

All women were informed about the research and they accepted the sampling of blood. Permission of the Bioethics Committee was also obtained.

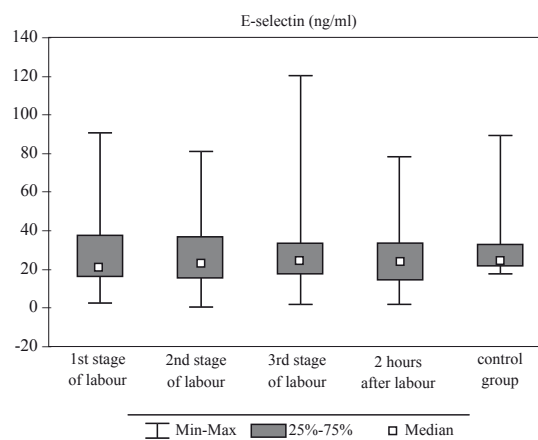
### Sampling of the blood

Blood samples were obtained from antecubital vein in the 1st, 2nd and 3rd stages of labour, as well as two hours after placenta expulsion. 3.2% sodium citrate was used as an anticoagulant in the proportion 1:9 (one part of 3.2% sodium citrate, nine parts of the blood). The blood was placed in a plastic test-tube and taken to the lab to be centrifuged ( $2500 \times g$ , 20 min,  $+4^\circ\text{C}$ ). The plasma was divided into 200  $\mu\text{l}$  portions which were closed tightly and stored for 3-5 weeks at  $-70^\circ\text{C}$ .

### Laboratory measurements

The concentration of soluble selectins E and P was measured by immunoenzymatic method (ELISA), using tests by Bender MedSystems. The manufacturer's instructions were strictly followed. The samples were assayed in batch operations. In detail: 10  $\mu\text{l}$  plasma was used to measure P-selectin, and 90  $\mu\text{l}$  assay buffer was added (procedure in duplicate); 20  $\mu\text{l}$  plasma was used to measure E-selectin and 80  $\mu\text{l}$  sample diluent with added. The interassay and intraassay coefficients of variability were  $<10\%$ .

**Figure 1. Plasma soluble endothelial selectin (E-selectin) at delivery and post partum**



### Statistical analysis

The Kolmogorov-Smirnov test was used to evaluate the data for normality. The data were not normally distributed and thus the medians and corresponding quartiles (lower – Q1, upper – Q3) are given. Statistical analysis was performed with two tests: (i) The ANOVA Friedman's test was used for comparison of the data of labour stages; (ii) Non-parametric test of Mann-Whitney was used for evaluation of unpaired data (parturient women vs control group). The significance limit was chosen at  $p$  value  $<0.05$ .

## Results

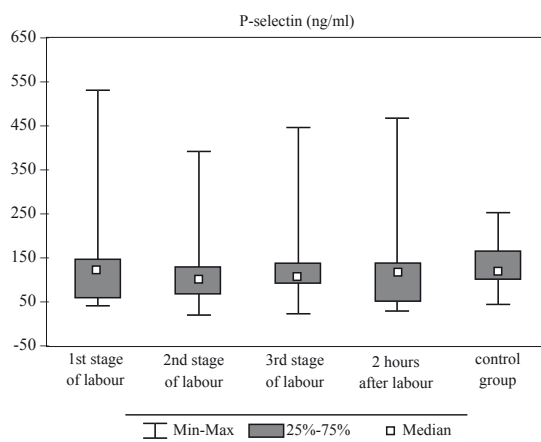
**E-selectin:** The medians and quartiles (Q1-Q3) of the level of E-selectin in blood plasma of labouring women were as follows: in the 1st stage of labour – 23.50 ng/ml (15.80-38.03 ng/ml), in the 2nd stage – 23.90 ng/ml (16.05-37.25 ng/ml), in the 3rd stage – 24.80 ng/ml (17.30-32.80 ng/ml), and two hours after labour – 22.40 ng/ml (11.75-32.00 ng/ml). In control group (non-labouring women at term) the median was 25.67 ng/ml (22.95-33.35 ng/ml). The differences between labouring women and controlled women were not significant ( $p>0.05$ ) (Fig. 1).

**P-selectin:** The medians and quartiles (Q1-Q3) of plasma level of P-selectin in blood plasma of labouring women were as follows: in the 1st stage of labour – 117.03 ng/ml (63.66-147.27 ng/ml), in the 2nd stage – 101.90 ng/ml (75.58-127.11 ng/ml), in the 3rd stage – 107.66 ng/ml (91.10-141.51 ng/ml), and two hours after labour – 106.94 ng/ml (47.77-137.19 ng/ml). In the control group (non-labouring women at term) the level was 116.70 ng/ml (91.82-179.00 ng/ml). The differences between labouring women and controlled women were not significant ( $p>0.05$ ) (Fig. 2).

## Discussion

In our working hypothesis we turned out to be wrong to assume a possibility of change of the level of E- and/or P-selectin in blood plasma under the influence of labour itself. The

**Figure 2. Plasma soluble platelet selectin (P-selectin) at delivery and post partum**



levels of both molecules were stable in pre-labour time and at delivery as well as two hours after labour. Therefore we have concluded that the labour itself does not influence the levels of soluble E-selectin and soluble P-selectin in blood plasma. If it is the case, one can believe that these selectins are reliable biomarkers for supervision of preeclamptic patients during labour.

However, different results could be expected, because in the 3rd stage of labour two events take place which could – we thought – influence the level of examined biomarkers of plasma: (i) ablation of placenta which is accompanied by the local destruction of tissues including damage of vascular endothelium, and (ii) haemostasis of placental bed, which proceeds with the participation of blood platelets and their activation.

We can not compare our research with that of other authors, as they have not studied either E-selectin or P-selectin during labour, but in pregnancy complicated with preeclampsia. Most often they have studied these two molecules together with other adhesion molecules, like VCAM (vascular cell adhesion molecule) and ICAM (intercellular adhesion molecule) or others. Austgulen et al. [7] found increased levels of ICAM-1, VCAM-1 and E-selectin. Daniel et al. [3], who studied all three selectins, concluded that only the level of E-selectin shows statistically significant increase in preeclampsia. In contrast, Halim et al. [4] found out elevated level of P-selectin, and Chaiworapongsa et al. [5] reported a significant increase both in P-selectin and E-selectin, but decrease in L-selectin. Austgulen et al. [7], Krauss et al. [6] and Kim et al. [8] observed increased levels of VCAM, ICAM and E-selectin, and Besinger et al. [10] increased levels of E-selectin, pregnancy-associated plasma protein A (PAPP-A) and activin A. In pregnancy induced hypertension (PIH) the levels of E-selectin, thrombomodulin and von Willebrand factor (vWF) were also elevated [9].

Among the authors mentioned above, only Austgulen et al. [7] drew attention to the possibility of association of increase of ICAM and VCAM levels with the delivery. No changes were observed at delivery, but it is necessary to emphasize that the examined period of labour was not specified.

A question arises whether other known preeclampsia biomarkers do not lose their predictive values in the time of labour? The answer to that question would have a practical aspect facilitating intrapartum supervision of preeclamptic patients. Thus, it is neces-

sary to do further research of all markers throughout pregnancy and all stages of labour for preeclamptic women.

We consider our present study a preliminary report. We think that similar studies should include a group of preeclamptic women, whose labour will proceed naturally, the ways and powers of nature.

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