# Alteration of circulating placental leucine aminopeptidase (P-LAP) activity in preeclampsia

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**Abstract**

**OBJECTIVE:** Placental Leucine Aminopeptiadse (P-LAP) also known as oxytocinase, is secreted by syncytiotrophoblast and increases gradually during pregnancy until delivery. It is a regulator of uterine contractions, of vascular resistance and of volume of the retroplacental blood pool. Recently, it was shown that it could also regulate metalloproteinase 9 activity and thus, invasiveness of trophoblastic cells. Since development of preeclampsia could be initiated by decreased cytotrophoblastic invasion of spiral arterioles and a reduced uteroplacental perfusion, we speculate that circulating P-LAP activity could be decreased during preeclampsia.
**MATERIALS AND METHODS:** Case-control study was evaluated in 84 women. P-LAP activity was measured in n=51 healthy pregnant women at term, and compared with n=16 normotensive women delivering preterm and n=17 women diagnosed with pre-eclampsia. P-LAP activity was determined by colorimetry in plasma samples using L-Leucine-p-nitroanilide as substrate.
**RESULTS:** P-LAP activity was significantly lower in sera of preeclamptic women (0.91±0.122 mDO/min) as compared to normotensive controls (1.41±0.103 mDO/min; $p$=0.003) irrespective of time of delivery.
**CONCLUSIONS:** These findings confirm the probable involvement of P-LAP in trophoblast invasion and development of preeclampsia.

## INTRODUCTION

Preeclampsia (PE) is a common disorder affecting 5 to 8% of all pregnancies. It is characterized by maternal hypertension, proteinurea and edema. It represents a major cause of maternal and foetal morbidity and mortality, and is often associated with substantial health problems later in life in both women and their child. The initiating event of PE could be due to a reduced uteroplacental perfusion leading to an abnormal cytotrophoblast invasion of spiral arterioles (Robertson *et al.* 1967; Gerretsen *et al.* 1981). Mechanisms involved in shallow invasion or that govern trophoblastic differentiation in preeclampsia are still unknown but it has been postulated that factors regulating trophoblast invasion could be used as markers of preeclampsia. Among these factors, matrix metalloproteinases (MMPs) 2 and 9, by their capacity to degrade matrix components, are considered to be instrumental in trophoblastic invasion (Cohen *et al.* 2006). Several studies focused on these factors and a decreased activity of these proteases has been described in women diagnosed with PE (Myers *et*



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*al.* 2005; Pang *et al.* 2003; Narumiya *et al.* 2001), while this has not been confirmed in a recent study (Palei *et al.* 2008). These differences could be due to differences in preanalytic conditions that modify the results of quantification of MMPs in serum (Meisser *et al.* 2005).

We recently determined that P-LAP is a potent up regulator of MMP-9 activity, and thus of trophoblastic invasion (Cohen *et al.* 2008). This protease had already been described as a potent regulator of trophoblast invasion (Graham *et al.* 1996), and a potent marker for PE (Mizutani *et al.* 1985; Ichaliotis *et al.* 1964). However, Mizutani et al. described an increase of P-LAP activity in serum of PE patients compared to normal patients whereas Ichaliotis and Lambrinopoulos observed the opposite. If we consider that P-LAP could play a role in trophoblastic invasion, we suggest that it could be decreased during PE. The aim of this study was to determine if P-LAP activity is at all altered in PE independently of time of delivery.

## MATERIAL AND METHODS

After IRB approval and written informed consent, 84 women were recruited prospectively between 12 weeks and term. At the time of delivery, a venous peripheral blood sample was collected from 51 healthy pregnant women at term (controls), 16 normotensive pregnant women delivering preterm (preterm) and 17 women diagnosed with preeclampsia according to ACOG criteria (13 diagnosed with severe PE, and 4 diagnosed with mild PE; ACOG Practice Bulletin, 2002). Because P-LAP activity increases with gestational age, preterm patients have chosen with a time of delivery comparable to time of delivery of preeclamptic patients.

**Tab. 1.** Characteristics of the control and PE groups.

| At delivery | Pre-eclamptic group | Control group | *t-student* |
|---|---|---|---|
| **Nulliparus** (%) | 80.9 | 100 | 0.08 |
| **Weight gain** (kg) | 14.8±7.7 | 18.2±1.0 | 0.174 |
| **BMI** | 31.9±7.5 | 29.2±4.6 | 0.23 |
| **Gestational age** | 35.3±3.3 | 40.2±0.2 | 0.0001 |
| **Birthweight** (g) | 2393.8±861 | 3346.6±146 | 0.003 |

**Tab. 2.** Characteristics of Preterm and PE groups.

| At delivery | Pre-eclamptic group | Preterm group | *t-student* |
|---|---|---|---|
| **Nulliparus** (%) | 80.9 | 100 | 0.08 |
| **Weight gain** (kg) | 14.8±7.7 | 15.7±1.4 | 0.708 |
| **Gestational age** | 35.3±3.3 | 36.2±0.8 | 0.386 |
| **Birthweight** (g) | 2393.8±861 | 2484.7±222 | 0.757 |

Leucine aminopeptidase activity was measured using L-Leucine-p-nitroanilide as a substrate in presence of 20mM L-Methionine. Briefly, leucine aminopeptidase by hydrolysing the substrate liberates p-nitroanilide that is measured in a spectrophotometer as optical density (OD) at 405 nm. The subtsrate solution was prepared in a NaCl 140mM and HCl 50mM Tris Buffer at pH 7.4. The L-Leucine-p-nitroanilide and L-Methionine were from Sigma (Sigma-Aldrich, Buchs, Switzerland). Samples were loaded in duplicates in a 96 well plate. For each sample, the OD was determined at 30, 60, 90, and 120 minutes and after substraction of the blank (OD measured at t=0), the value was divided by the time to yield a mean OD/min. For each sample, a mean OD/min was established. A ceiling in leucine aminopeptidase activity was reached after 60 minutes.

Data were reported as means ± SEM. The comparisons of groups were assessed by bilateral Student's t test. A probability value $< 0.05$ was considered significant.

## RESULTS

Clinical characteristics of the study population are shown in table I. Weight gain and body mass index was not different between the groups (BMI, Table 1). As expected gestational age at delivery and neonatal birth weight were significantly different between the groups. Because P-LAP activity increases with gestational age, we then compared patients with preterm delivery comparable to time of delivery of preeclamptic patients (n=17, Table 2) with PE patients.

We found a significant difference of P-LAP activity between the normotensive women (controls) and preeclamptic women. P-LAP activity was significantly lower in plasma of preeclamptic women as compared to controls delivering at term or to controls matched to gestational age (preterm) (Figure 1). In contrast, the activity of P-LAP of the controls delivering at term was not significantly different from P-LAP activity in plasma of preterm controls ($p$=0.723, Figure 1). Despite the number of patients with mild PE is not sufficient to conclude on P-LAP activity value, it seems that P-LAP activity in plasma of women diagnosed with mild PE is not different of P-LAP activity in plasma of control women (Figure 2).

## DISCUSSION

Our findings demonstrate that P-LAP activity is altered in preeclamptics, regardless of gestational age at the time of delivery.

P-LAP also called oxytocinase, is widely distributed and not specific to placenta as originally thought. It was found to be able to hydrolyse small peptides such as oxytocin, vasopressin, angiotensin III, somatostatin, Lys-bradykinin, Met-enkephalin, dynorphin A and nurokinin A (Matsumoto *et al.* 2000; Tsujimoto *et al.* 1992; Herbst *et al.* 1997; Matsumoto *et al.* 2001). In pla-

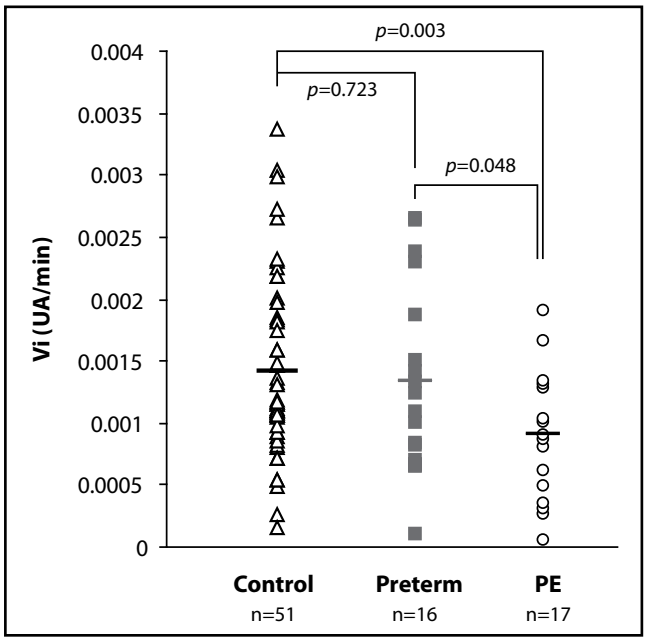


**Fig. 1.** Distribution of P-LAP activity in control, preterm and PE groups.

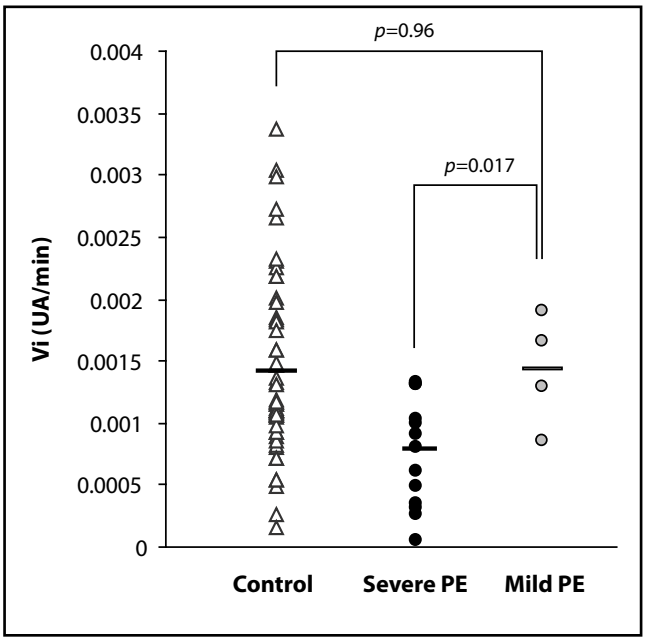


**Fig. 2.** Distribution of P-LAP activity in control, severe PE and Mild PE groups.

cental tissue, P-LAP is present in a membrane-bound form. Its mRNA is mostly expressed in syncytiotrophoblast (Yamahara *et al.* 2000; Nagasaka *et al.* 1997), but it is also highly expressed in extravillous cytotrophoblastic cells invading the decidua or maternal spiral arteries (Ino *et al.* 2003). P-LAP is also found in a truncated form in serum of pregnant women suggesting that this enzyme originates from placenta (Yamahara *et al.* 2000; Nagasaka *et al.* 1997). The fact that its level has been shown to increase with advancing gestation both in maternal serum and in placenta, reaching a peak at 37 weeks (Yamahara *et al.* 2000) and disappearing after delivery further confirms its placental origin.

Many studies investigated the regulation of P-LAP gene expression in trophoblast. Activator protein-2 (AP-2) was shown to be the main activator of P-LAP promoter (Nomura *et al.* 2005), and Ikaros could cooperate with this transcription factor for maximal expression of P-LAP gene (Yamamoto *et al.* 2005). Ikaros is predominantly expressed in extravillous cytotrophoblast cells and the suppression of Ikaros transcriptional function in these cells lead to a decrease in migration and invasion of extravillous cytotrophoblastic cells. This observation suggests a possible role of its transactivator in migration and invasion of trophoblastic cells in early placentation. In parallel, the increase in P-LAP expression during placentation and in the invasive tumor cell of trophoblast suggests an involvement of this peptidase in invasiveness of normal and malignant trophoblasts (Ino *et al.* 2003). We recently confirmed that P-LAP could regulate invasive properties of cytotrophoblastic cells, probably by down-regulation of MMP-9 activity which is an instrumental enzyme for trophoblast invasion (Cohen *et al.* 2008). There is substantial evidence to suggest that P-LAP could play an important role in regulation of invasive properties of trophoblastic cells. Furthermore, the fact that its activity is decreased in severe PE is in agreement with the study of Ichaliotis and Lambrinopoulos (Ichaliotis *et al.* 1964), and with the hypothesis that shallow trophoblast invasion of the spiral arteries contributes to the development of PE. Interestingly, this peptidase also plays an important role in degradation of different pressor hormones and peptides as vasopressin, angiotensin peptides, hemorphins and thus, could be involved in the pathogenesis of hypertensive disorders in pregnancy.

While decreased P-LAP activity underscores the instrumental role of trophoblastic invasion, it remains to be established if this parameter could have any predictive value earlier in pregnancy to detect PE and could serve as a therapeutic target.

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