# The possible role of the PK1 and its receptor in the etiology of the preeclampsia

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**Abstract**

**OBJECTIVE:** Preeclampsia (PE) is a hypertensive complication of the pregnancy. In our study we investigated the expression, localization, and signaling pathways of PK1 and PKR1 in third-trimester human placenta and myometrium and assessed the correlation between the PK1 and PKR1 expression and signaling and the incidence of the PE.

**MATERIALS & METHODS:** We designed two study groups: pregnant PE patients and healthy, pregnant women. After collection, tissue was placed in RNAlater for RNA extraction, fixed in 4% neutral buffered formalin, and wax embedded for immunohistochemistry or placed in RPMI and transported to the laboratory for *in vitro* culture. We have collected and processed placental and myometrial biopsies from 40 patients – 19 were PE patients.

**RESULTS:** Only the PK1 mRNA expression comparison between PE and CTRL in placenta showed statistically significant difference ($p$=0.004). There was statistically significant difference in cell signaling in myometrium controls in 30 minutes after ligand. The rise in pERK/tERK ratio is clearly visible in time intervals 20 and 30 minutes in controls, although with no statistical significance. There was no difference in PK1 and PKR1 localization in the placenta and myometrium in the groups.

**CONCLUSIONS:** The number of PKR1 is not reduced in PE. The reduced PK1 mRNA expression is not than dependent on PKR1 mRNA expression. The data shows that ST produces much more PK1 in healthy pregnant women than those suffering from PE. We can conclude that in PE the production of PK1 is impaired and so are the endocrine functions of the ST.

ORIGINAL ARTICLE

## INTRODUCTION

Preeclampsia (PE) is a hypertensive complication of 5–7% (Walker 2000) of all pregnancies worldwide, is responsible for approximately 15% of preterm deliveries and is associated with significant perinatal morbidity and mortality of the newborns (Kanasaki & Kalluri 2009). PE increases the future risk of developing hypertension, coronary heart disease and cerebro-vascular disease in mothers. In spite of recent findings, the patophysiology of PE still remains unclear. The placenta developing from the trophoblast, seems to be the organ in which the pathology starts, as the presence of

the placenta, but not necessarily the fetus is essential to develop the PE. Moreover, the only way to entirely treat the PE is the labour, in which the placenta is being delivered from the uterus (Khong 1991).

So far, the most up-to-date hypothesis of the origin of the preeclampsia was the two-stage hypothesis (Khong 1991). The preclinical first stage takes place early in the pregnancy, when there is improper invasion of the trophoblast leading to the impaired development of the placenta, and further to its ischaemia (Kobashi 2004). The second stage is when the oxidative stress placenta release to the maternal circulation factors inducing systemic inflammatory response and endothelial dysfunction, thus producing clinical symptoms of the PE (Huppertz 2008).

The improper invasion of the trophoblast into the wall of the spiral arteries is, regardless of the etiology, the basic mechanism responsible for the preeclapmsia. The consequence of the invasion is the remodeling of the vessels into thin-walled, wide-open structures, which lowers systemic vascular resistance, thus contributes to feto-maternal exchange. Unfortunately, the improper invasion of the trophoblast cannot explain alone the origin of the preeclampsia, as the same pathology is found in the intrauterine growth restriction of the fetuses without any typical for PE signs. Therefore, it has been proposed that in the etiology of the preeclampsia the presence of the maternal factors predisposing to the vascular changes is essential (Khong 1991).

Prokineticin-1 (PK1) is a recently identified protein with a range of physiological and pathological functions (Maldonado-Perez *et al.* 2007). This peptide, also known as endocrine gland derived vascular endothelial growth factor (EG-VEGF), is expressed in steroidogenic tissues including the placenta (LeCouter *et al.* 2001, Hoffmann *et al.* 2006), ovary (Ferrara 2003, Fraser 2005) and adrenal (Lin R 2002). It mediates tissue specific vascular effects, which include capillary endothelial cell survival, proliferation, differentiation, and induction of fenestrae (Lin R 2002). PK1 shares two cognate G-protein coupled receptors (GPCR) – prokineticin receptor 1 (PKR1) and 2 (PKR2) (Lin DC 2002, Masuda 2002). These couple to either Gi or Gq (Lin DC 2002, Lin R 2002, Soga 2002), activating downstream signaling pathways that include calcium mobilization, stimulation of phosphoinositide turnover, and activation of MAPK.

Recently, a new hypothesis has been proposed, suggesting important role of PK1 and PKR1 in human placentation, mostly because of its peaking expression correlating with the hypoxic period in placenta l development, before the villi circulation being established (Hoffmann *et al.* 2006). Strong expression of the PK1 in syncytiotrophoblast, which is regarded as an endocrine component of the placenta, suggests that the degree of the expression may be correlated with the degree of the placenta differentiation. Hence the PK1 is being regarded as a novel placental growth factor (Hoffmann *et al.* 2006), thus it may play an important role in the etiology of preeclampsia.

Therefore in our study we set up two objectives: first, to investigate the expression, localization, and signaling pathways of PK1 and PKR1 in third-trimester human placenta and myometrium and second to assess the correlation between the PK1 and PKR1 expression and signaling and the incidence of the PE.

## MATERIALS AND METHODS

*Patients and tissue collection*

The study had taken place in Medical Research Council, Human Reproductive Sciences Unit, Centre for Reproductive Biology, University of Edinburgh and was subsequently followed in the Department of Perinatology and Gynaecology, University of Medical Sciences in Poznan, Poland. The same inclusion, exclusion criteria, as well as protocols were used. We designed two study groups: pregnant PE patients (PE was defined as an onset of hypertension from 20$^{th}$ week of gestation until the 2$^{nd}$ week of puerperium) with systolic – diastolic BP more than 140/90 mmHg on at least two occasions and urinary protein more than ++ on dipstick (or more than 0.5 g/24 H, or more than 300 mg/L) and otherwise healthy, pregnant women.

The samples were taken from placenta during the elective caesarean sections of the consented patients from PE group and controls (third trimester only). Shortly after collection, tissue was placed in RNALater (Applied Biosystems, Warrington, UK) for RNA extraction, fixed in 4% neutral buffered formalin, and wax embedded for immunohistochemistry or placed in RPMI (Sigma, Poole, UK) (containing 2 mm L-glutamine, 100 IU penicillin, and 100 µg/ml streptomycin) and transported to the laboratory for *in vitro* culture. Collection of samples was approved by the appropriate Ethics Comittees. We have collected and processed placental and myometrial biopsies from 40 patients. Among those 19 were PE patients.

*RNA isolation and RT-PCR analysis*

Tissue was placed in RNALater (Qiagen, Ambion) until RNA isolation. RNA isolation was conducted using Rneasy Mini Kit (Qiagen) according to the manufacturer's instructions. QiaShredder (Qiagen) homogenization columns were used to homogenize the samples, during the isolation the DNA elimination was conducted by using RN-ase Free Dnaze Set (Qiagen). Water solution of the isolated RNA was frozen at 20 °C. The reverse transcription was conducted by using DYnamo Syber Green™ 2-step qPCR (Finnzymes, Finland). According to the manufacturers protocols, 1 µl of previously isolated RNA, containing <1 µg RNA and starter oligodT for mRNA was used. The quantitative assessment of the transcript level for PK1 and PKR1 was conducted by using Real-time PCR. Starters to the reaction were designed using software Primer3 on the

basis of the mRNA sequence, in FASTA format received from NCBI-EntrezGene database. Thermodynamic parameters were verified by using OligoAnalizer1.2 software and specifity of the starters were compared in BLAST database. Starters melting temperature was established by using Finnzymes calculator and was the extraction point to determine annealing temperature for particular transcripts. The efficacy of the reaction and standard curve was determined in qPCR reaction, for which six subsequent dilutions of the linear form of the specific for the starters product were the matrix. The amount of the transcript was determined with reference to inner marker – the gene of the constitutive for GAPDH and α-actin expression. The reaction was carried out by using DYnamo Syber Green™ 2-step qPCR reagents (Finnzymes, Finland), according to the manufacturer's recommendations. The reaction was conducted double for all tests. Received CT for particular tests was referred to the standard curve. The relative expression was determined dividing the values taken from the standard curve for PK1 and PKR1 transcripts over the reference genes GAPDH and α-actin values.

*Cell signaling analysis*

To investigate PKR1 signaling pathways in placenta and myometrium, after washing in PBS, finely chopped (2–3 mm) tissue was incubated overnight at 37 °C in serum-free medium [DMEM/F-12 medium with L-glutamine (Invitrogen) containing 100 IU penicillin and 100 µg/ml streptomycin (PAA Laboratories, Middlesex, UK)]. Tissue explants were then incubated for 0, 5, 10, 20, and 30 min in the presence of 40 nmPK1 (Peprotech, London, UK), snap frozen in dry ice, and stored at –80 °C degrees for protein analysis. Frozen tissue fragments were lysed and proteins extracted in 750 ml lysis buffer containing 150 mm NaCl, 50 mm Tris-HCl (pH 7.4), 10 mm EDTA, 0.6% Nonidet-P40 substitute, and 10% glycerol containing protease inhibitors, in a tissue lyser (QIAGEN, Crawley, UK) and centrifuged for 20 min at 19,000 × g. Proteins were quantified by the method of Lowry (Bio-Rad, Hemel Hempstead, UK).

*Western blot analysis*

To investigate phosphorylation of ERK1/2, proteins (20–40 g) were resolved on 4–12% Bis-Tris gels (Nupage; Invitrogen) and transferred onto polyvinylidene difluoride membrane (Millipore, Watford, UK). Membranes were blocked for 1 h at 25 °C in Odyssey blocking buffer (Li-Cor Bioscience, Cambridge, UK) and incubated overnight at 4 C in blocking buffer containing rabbit anti-phospho-p42/44 ERK and mouse anti-p42 ERK (both obtained from Cell Signaling Technologies, New England Biolabs, Hertfordshire, UK; 1:1,000). After washing in PBS containing 0.05% Tween, membranes were incubated for 1 h at 25 °C C degrees in the dark in antimouse IgG conjugated to IRDYE 800 (Tebu-bio, Peterborough, UK) and antirabbit IgG conjugated to Alexa Fluor 680 (Invitrogen) both at a dilution of 1:5,000 in blocking buffer. Membranes were washed and proteins visualized and quantified using an Odyssey Infrared Imaging System (Li-Cor Bioscience). Relative density of immunoblots was calculated as phosphorylated ERK divided by the total ERK and was expressed as a fold change above vehicle controls.

*Immunohistochemistry*

PK1 and PKR1 were localized by immunohistochemistry as previously described (Li *et al.* 2001, Lin DC *et al.* 2002). Briefly, 5-µm placental sections were dewaxed in xylene and rehydrated in graded ethanol. Antigen retrieval was performed by treating sections for 5 min in a pressure cooker in boiling 0.01 M citrate buffer (pH 6.0). Endogenous peroxidase activity was quenched with 10% $H_2O_2$ in methanol at room temperature. Nonimmune swine serum (20% serum in Tris-buffered saline) was applied for 30 min before overnight incubation at 4 °C with rabbit antihuman PK1 (1:1,000; Phoenix Pharmaceuticals Inc., Belmont, CA) or rabbit antihuman PKR1 (1:500; Caltag Medsystems, Botolph Claydon, UK). An avidin-biotin peroxidase detection system was then applied (Dako Ltd., Cambridge, UK) with 3,3-diaminobenzidine as the chromogen. Controls were incubated with isotype-matched IgG in place of primary antibody. Ultimately, the total number of 38 placenta and myometrium samples were examined.

*Statistics*

The mRNA expression and cell signaling data were subjected to statistical analysis by using Mann-Whitney test and in addition the Spearmann test was used to analyze the correlation of the time dependence in cell signaling. The statistical significance was accepted when $p<0.05$.

## RESULTS

*PK1 and PKR1 mRNA expression in placenta and myometrium*

The following Table 1 shows the values of the PK1 and PKR1 expression in placenta and myometrium in groups of the patients with pregnancy complicated by PE and in controls (CTRL). The groups with the random distribution of the feature were analyzed, so for statistics the non-parametric statistics were used. The numerical values of the expression refer to the reference i.e. expression of PK1 and PKR1 in normal, eutopic endometrium, in 2nd phase of the menstrual cycle.

The following Tables 2–5 and Figures 1–4 compare PE and control group in terms of the statistical significance of the difference in the PK1 and PKR1 mRNA expression.

*Cell signaling*

The following Table 6 compares the correlating time intervals in patients with preeclampsia (PE) and con-

**Tab. 1.** The values of the PK1 and PKR1 expression in placenta and myometrium in groups of the patients with pregnancy complicated by PE and in controls (CTRL).

| Group | n | Mean | Std Dev | Std. Error | Max | Min | Median | 25% | 75% |
|---|---|---|---|---|---|---|---|---|---|
| Placenta CTRLPKR1 | 20 | 3.215 | 9.242 | 2.067 | 41.429 | 0.0373 | 0.475 | 0.114 | 1.626 |
| Placenta PKR1 | 12 | 0.793 | 0.44 | 0.127 | 1.534 | 0.131 | 0.934 | 0.39 | 1.019 |
| MyoCTRLPKR1 | 13 | 3.335 | 6.266 | 1.738 | 20.072 | 0.0266 | 0.286 | 0.134 | 2.646 |
| MyoPKR1 | 11 | 0.328 | 0.34 | 0.103 | 1.232 | 0.0531 | 0.218 | 0.109 | 0.371 |
| PlacentaCTRLPK1 | 20 | 5.655 | 10.404 | 2.326 | 47.684 | 0.0847 | 2.041 | 1.325 | 5.933 |
| PlacentaPK1 | 12 | 1.056 | 1.438 | 0.415 | 5.222 | 0.106 | 0.581 | 0.171 | 1.265 |
| MyoCTRLPK1 | 13 | 3.874 | 10.084 | 2.797 | 36.405 | 0.0816 | 0.337 | 0.182 | 0.808 |
| MyoPK1 | 11 | 2.48 | 6.891 | 2.078 | 23.241 | 0.026 | 0.359 | 0.159 | 0.727 |

**Tab. 2.** The comparison of the PKR1 mRNA expression between PE and CTRL in placenta using Mann-Whitney test showed no statistically significant difference ($p$=0.471).

| Mann-Whitney Rank Sum Test | | | | | |
|---|---|---|---|---|---|
| **Normality Test:** | **Failed** | **($p$<0.05)** | | | |
| **Group** | **N** | **Missing** | **Median** | **25%** | **75%** |
| Placenta CTRLPKR1 | 20 | 0 | 0.475 | 0.114 | 1.626 |
| Placenta PKR1 | 12 | 0 | 0.934 | 0.39 | 1.019 |
| ($p$=0.471) | | | | | |

**Tab. 3.** The comparison of the PKR1 mRNA expression between PE and CTRL in myometrium using Mann-Whitney test showed no statistically significant difference ($p$=0.487)

| Mann-Whitney Rank Sum Test | | | | | |
|---|---|---|---|---|---|
| **Normality Test:** | **Failed** | **($p$<0.05)** | | | |
| **Group** | **N** | **Missing** | **Median** | **25%** | **75%** |
| MyoCTRLPKR1 | 13 | 0 | 0.286 | 0.134 | 2.646 |
| MyoPKR1 | 11 | 1 | 0.218 | 0.109 | 0.371 |
| ($p$=0.487) | | | | | |

**Tab. 4.** The comparison of the PK1 mRNA expression between PE and CTRL in placenta using Mann-Whitney test showed statistically significant difference **($p$=0.004).**

| Mann-Whitney Rank Sum Test | | | | | |
|---|---|---|---|---|---|
| **Normality Test:** | **Failed** | **($p$<0.05)** | | | |
| **Group** | **N** | **Missing** | **Median** | **25%** | **75%** |
| PlacentaCTRLPK1 | 20 | 0 | **2.041** | 1.325 | 5.933 |
| PlacentaPK1 | 12 | 0 | **0.581** | 0.171 | 1.265 |
| ($p$=0.004) | | | | | |

**Tab. 5.** The comparison of the PK1 mRNA expression between PE and CTRL in myometrium using Mann-Whitney test showed no statistically significant difference ($p$=0.685).

| Mann-Whitney Rank Sum Test | | | | | |
|---|---|---|---|---|---|
| **Normality Test:** | **Failed** | **($p$<0.05)** | | | |
| **Group** | **N** | **Missing** | **Median** | **25%** | **75%** |
| MyoCTRLPK1 | 13 | 0 | 0.337 | 0.182 | 0.808 |
| MyoPK1 | 11 | 1 | 0.359 | 0.159 | 0.727 |
| ($p$=0.685) | | | | | |

trols (CTRL). The Mann-Whitney test was used, as the proportions were analyzed and the data distribution was non-parametric. Therefore medians, not the mean values were analyzed. The following Figures 5–8 show relative levels of intracellular signaling in myometrium and placenta in both groups.

There was statistically significant difference in myometrium controls in 30 minutes after ligand stimulation (EG-VEGF/PK1). There was no statistically significant difference between other groups, although the rise in pERK/tERK ratio is clearly visible in time intervals 20 and 30 minutes in controls.

In the Table 7 we have shown the analysis of the correlation of the levels pERK/tERK ratio with time intervals using Spearman test. In the controls rise in pERK/tERK ratio correlated in time and correlation index was positive, so we can conclude that those variables were rising in time. In PE group there was no statistically significant difference ($p$-value > 0.05), so the variables didn't rise in time.

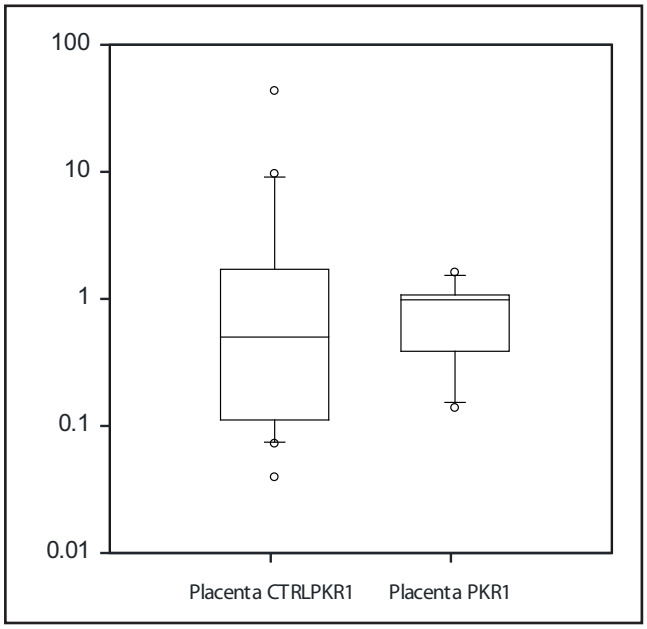


**Fig. 1.** The comparison of the PKR1 mRNA expression between PE and CTRL in placenta.

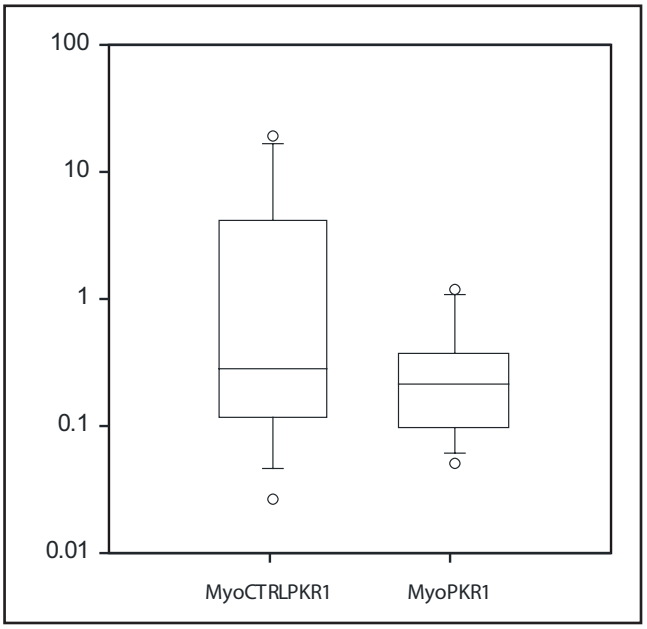


**Fig. 2.** The comparison of the PKR1 mRNA expression between PE and CTRL in myometrium.

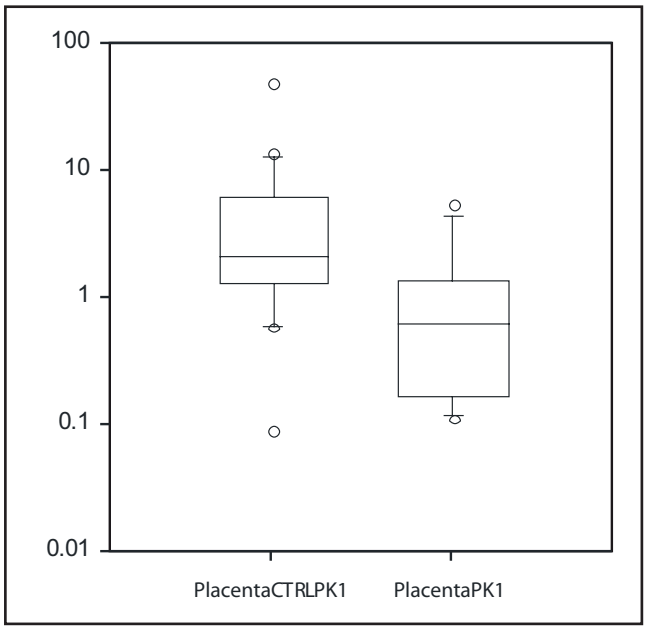


**Fig. 3.** The comparison of the PK1 mRNA expression between PE and CTRL in placenta.

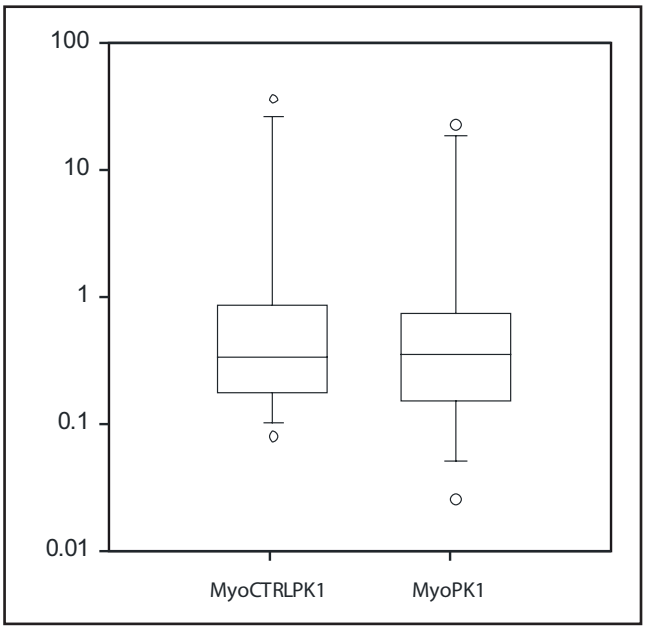


**Fig. 4.** The comparison of the PK1 mRNA expression between PE and CTRL in myometrium.

*Immunohistochemistry*

There was no difference in PK1 and PKR1 localization in the placenta and myometrium of the controls and PE patients. In placenta PK1 was immunolocalized mainly in the nuclei of the syncytiotrophoblast covering villi. Expression of this protein was strongest in nuclei, whereas in the cytoplasm there was only gentle discolouration. Comparable reaction was observed in mesenchymal cells of the villi stroma (Figure 9).

The PKR1 was mainly immunolocalized in the cytoplasm of the trophoblast cells, nuclei were negative. In the mesenchymal stroma of the villi the cytoplasm was colouring only single cells; probably Hofbauer cells (Figure 10).

In myometrium the PK1 was localized in the nuclei of the smooth muscle cells and in the nuclei of the stromal cells. The cytoplasm was negative (Figure 11), the nuclei of the cytotrophoblast of the placental bed

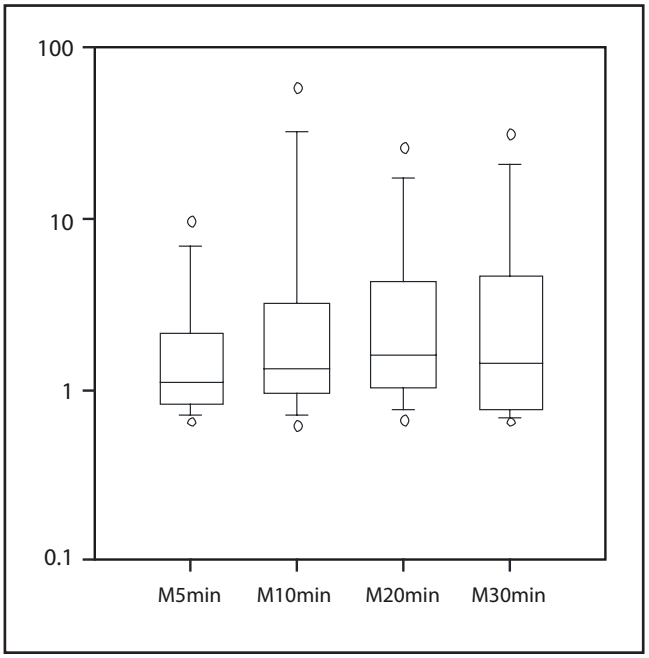


**Fig. 5.** Relative levels of the intracellular signaling in myometrium – PE

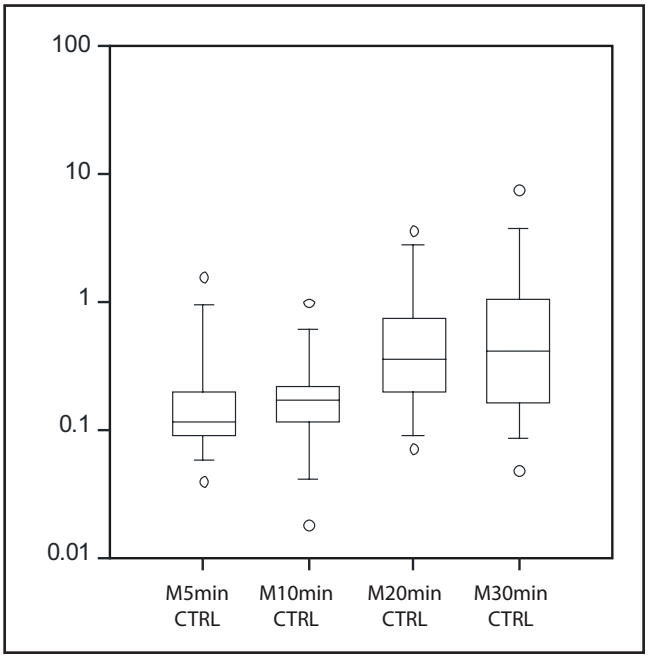


**Fig. 6.** Relative levels of the intracellular signaling in myometrium – CTRL

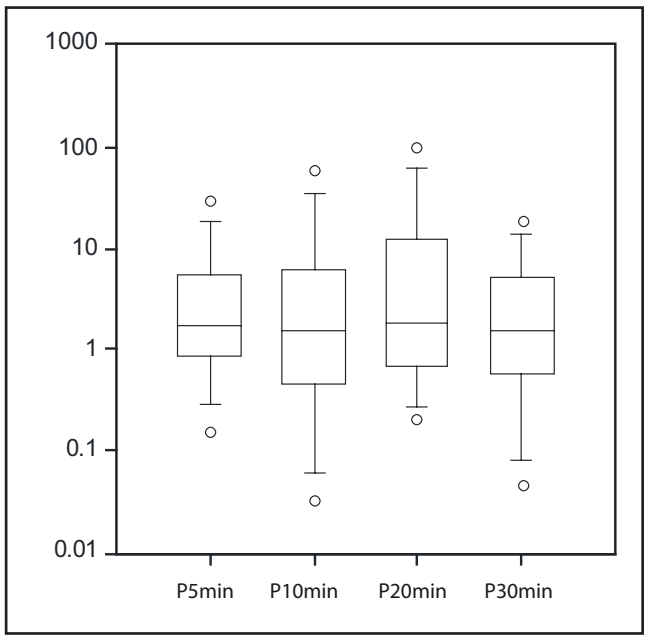


**Fig. 7.** Relative levels of the intracellular signaling in placenta – PE

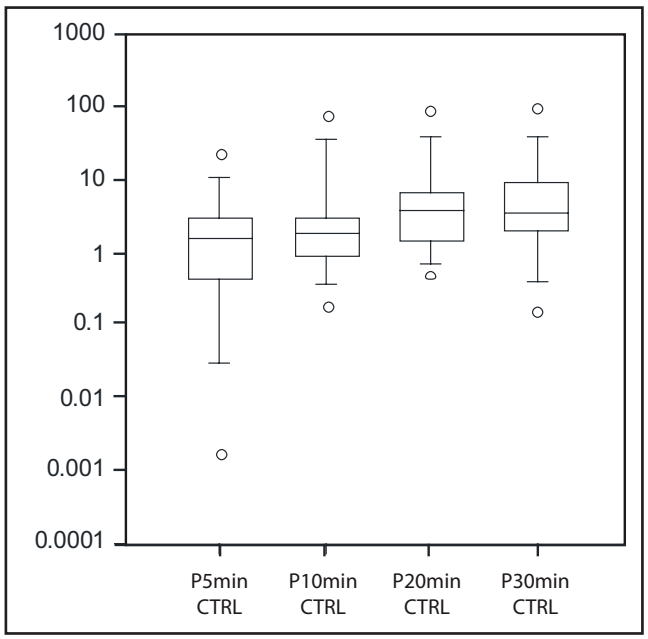


**Fig. 8.** Relative levels of the intracellular signaling in placenta– CTRL

and decidua were positive. The PKR1 expression in the myometrium was very weak and most often negative, whereas in the decidual plate the immunoreaction was positive (Figure 12).

## DISCUSSION

The etiology of the preeclampsia remains to be the subject of the extensive research over the recent years. Yet it is believed that it is multifactorial and the presence of the placenta rather than the fetus is responsible for the development of preeclampsia. Although the placenta plays a crucial role in the development of preeclampsia, the onset, severity, and progression is significantly affected by the maternal response to placentally derived factors and proteins (Huppertz 2008).

In the literature of the recent years there are data suggesting that the improper invasion of the trophoblast may be the main mechanism of this pathology. But the reason for the impaired invasion remains the subject of many controversies. The two-stage hypothesis was replaced by the three-stage hypothesis, yet it

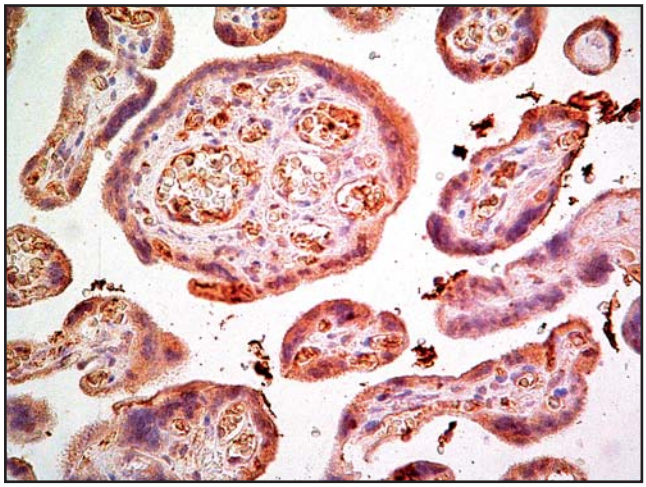

**Fig. 9.** The PK1 immunolocalized in syncytiotrophoblast with gentle discolouration of the cytoplasm (magnification 270×).

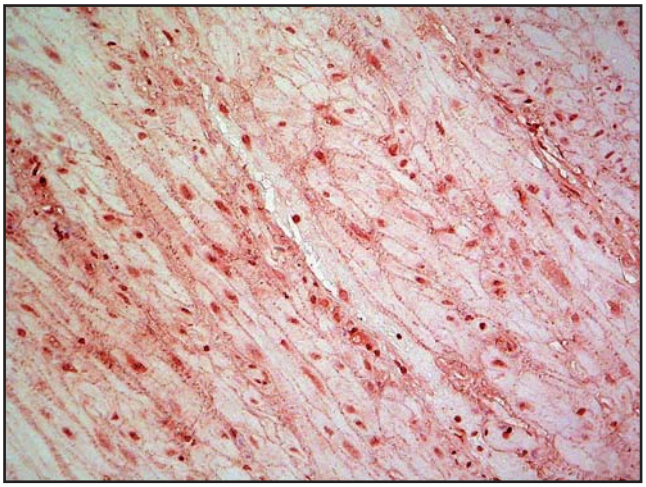

**Fig. 10.** The immunolocalization of the PKR1 in syncytiotrophoblast. The negative nuclei coloured violet-bluish with haematoxylin. The immunological reaction in the cytoplasm is yellow-brownish (magnification 270×).

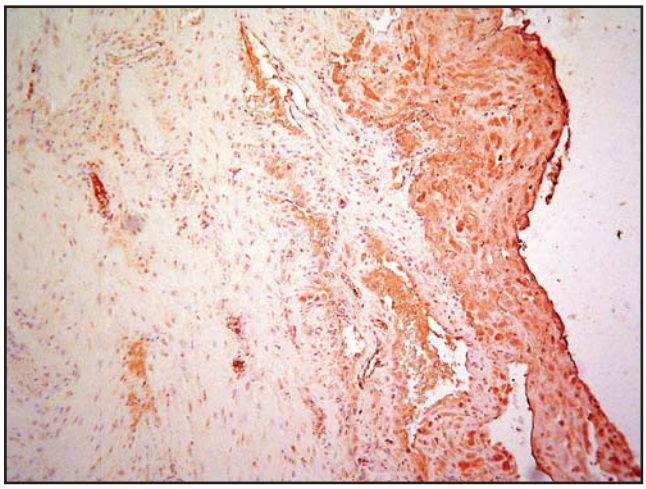

**Fig. 11.** The immunopositive reaction for PK1 in the nuclei of the myometrium smooth muscle cells (a – ×200).

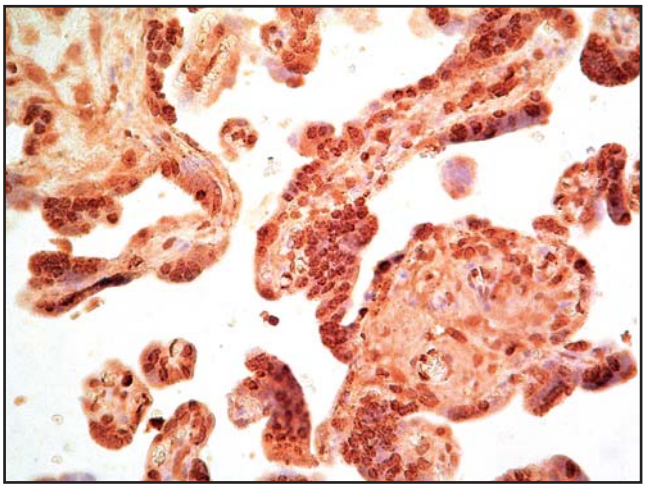

**Fig. 12.** The immunoreaction of the PKR1 in the myometrium. The sample from the uterine wall with decidua. In the smooth muscle reaction is negative, blue-violet haematoxylin discolouration of the nuclei is visible. In the decidua the positive immunoreactions is visible in the cytoplasm of the decidual cells (magnification 150×).

needed to be completed by the fourth stage, i.e. first in the chronological order – the genetical disturbances (Rasmussen & Irgens 2007).

Over the last few years there is growing evidence that preeclampsia is not homogenous in term of the course. There is a difference in etiology, course and prognosis depending on time of the onset of the pathology (von Dadelszen 2003). The preeclampsia can be divided into early-onset PE (before 34+0 weeks) and late-onset PE (Huppertz 2008, Valensise 2008). The late onset type of preeclampsia comprises more than 80% of all pre-eclampsia cases worldwide. The early onset type of pre-eclampsia comprises a small subset of all preeclampsia cases (5% to 20%, depending on the statistics), but comprises the most severe cases of respective clinical relevance. Most of the symptoms occurring in the early-onset preeclampsia are also common for another major pregnancy pathology, intrauterine growth restriction (IUGR). The typical features of early onset IUGR cases are an inadequate trophoblast invasion, an inadequate transformation of spiral arteries, followed by respective changes in the blood flow of the uterine arteries, alterations of the umbilical blood flow, and restrictions of fetal growth (Soga 2002). Moreover, using Doppler wave form analysis in uterine arteries as a predictor of the PE can predict only 33–40% of the cases (Nicolaides 2006; Pilalis 2007), whereas the incidence of the IUGR in 100% (Pilalis 2007). Therefore one should ask a question whether the above mentioned changes are really pathognomic for early-onset PE (Huppertz

**Tab. 6.** The comparison of the relative levels of the intracellular signaling in time intervals after 5, 10, 20, 30 minutes of the ligand stimulation (PK1) in the placenta (P) and myometrium (M) in CTRL and PE groups.

| | Column | *p*-value | Size | Median | 25% | 75% |
|---|---|---|---|---|---|---|
| 1 | M5minCTRL | NS | 15 | 1.159 | 0.951 | 1.932 |
| | M5min PE | NS | 14 | 1.104 | 0.817 | 2.076 |
| 2 | M10minCTRL | NS | 15 | 1.774 | 1.27 | 2.16 |
| | M10min PE | NS | 14 | 1.323 | 0.996 | 3.235 |
| 3 | M20minCTRL | NS | 15 | 3.624 | 2.098 | 7.646 |
| | M20min PE | NS | 14 | 1.596 | 1.04 | 3.791 |
| 4 | M30minCTRL | 0.042 | 15 | 4.259 | 1.808 | 10.83 |
| | M30min PE | NS | 14 | 1.404 | 0.759 | 4.536 |
| 5 | P5PinCTRL | NS | 16 | 1.562 | 0.488 | 2.909 |
| | P5min PE | NS | 14 | 1.719 | 0.887 | 5.341 |
| 6 | P10minCTRL | NS | 16 | 1.811 | 1.112 | 2.749 |
| | P10min PE | NS | 14 | 1.533 | 0.471 | 5.466 |
| 7 | P20minCTRL | NS | 16 | 3.787 | 1.518 | 6.764 |
| | P20min PE | NS | 14 | 1.789 | 0.703 | 9.235 |
| 8 | P30minCTRL | NS | 16 | 3.626 | 2.212 | 9.076 |
| | P30min PE | NS | 14 | 1.482 | 0.674 | 5.203 |

**Tab. 7.** The analysis of the correlation of the pERK/tERK ratio with time intervals (Spearman test).

| Spearman Rank Order Correlation | | | | |
|---|---|---|---|---|
| **Cell Contents** | **MCTRL vs. time** | **PCTRL vs. time** | **MPE vs. time** | **PPE vs. time** |
| Correlation Coefficient | 0.45 | 0.332 | 0.105 | –0.056 |
| *p*-value | **0** | **0.00753** | 0.441 | 0.67 |
| Number of samples | 60 | 64 | 56 | 60 |

2008)? Maybe the combination with the IUGR makes the early-onset PE so severe? Is there in fact any difference between early and late-onset PE?

Hence, Huppertz (2008) proposed a most up-to-date model of the pathogenesis of the PE, taking into consideration its onset, placental origin and different appearance time of the symptoms. The preeclampsia may be caused by the inner factors impairing early in the trophoblast differentiation and development of the villous syncytiotrophoblast – ST – (unlike in IUGR where the extravillous cytotrophoblast – EVC – is being impaired). Later in the pregnancy, the external factors such as diabetes, multiple pregnancy, anemia, high altitude, may results in increased placental weight and/or surface. This and also some maternal disturbances leading to the overloading or improper removal of the apoptotic nuclei of the ST may result in secondary necrosis and inflammatory response injuring maternal endothelium (Huppertz 2008). What is important, in the light of those findings the ST seems to be the key place in the etiology of the PE.

The placenta is regarded by many authors as one of the places, in which prokineticin1 is being synthesized, its production is most intensive in ST (Hoffmann *et al.* 2006), although new research (Denison 2008) suggests its presence also in cytotrophoblast – CT and occasionally in endothelium of the fetal vessels. Probably that is why only in the placenta the comparison of the mRNA expression between PE and control group reached in our data statistical significance ($p$=0.004). In other words, the PK1 mRNA expression in the placenta of healthy pregnant women was higher than in placentas from preeclamptic patients. There was no statistically significant difference for PKR1 between two groups. We can therefore conclude that the number of receptor places for PK1 is not reduced in PE. The reduced PK1 mRNA expression is not than dependent on PKR1 mRNA expression. The data obtained shows that ST produces much more PK1 in healthy pregnant women than those suffering from PE. On this basis we can conclude that in PE the production of PK1 is impaired and so are the endocrine functions of the ST. Whether this impairement is primary or secondary to PE needs to be elucidated. Some insight into this problem reveals Hoffmann *et al.* (2008). In this research he proved that the PK1 concentrations were highest at the beginning of the pregnancy, than falling down to the third trimester where its concentrations were comparable to those of the non-pregnant women, i.e. five-fold smaller. As for the effect of the PK1 on EVT migration, mostly by affecting PKR2 being favorably expressed on the surface of the EVT, the scientist ceased this process (70% versus 40%). Especially relevant was the ability of the EVT to differentiate into invasive phenotype. In Hoffmann *et al.* (2008) research there was nearly no invasion of the EVT taken from human villi explants to Matrigel. That would confirm the role of the PK1 as an inhibitor of the invasion, but stands in conflict with the same author's data suggesting the highest physiological PK1 levels in the maternal serum in the first trimester. In contrast to previous observation concerning PK1 concentrations stands observed by the same authors (Hoffmann 2008) reduced in the presence of the PK1 creation of the tube-like structures transforming later into vessels. Also reduced in the presence of PK1 mieloperoxidases (such as MMP-2 and MMP-9) production is in contrast to elevated in the first trimester PK1 concentrations. The results of our research are also in contradiction to Chung *et al.* (2004) who first explored PK1 mRNA expression in normal and preeclamptic patients. He didn't however discover any statistically significant difference in PK1 expression between PE and normals, but it was VEGF that was his main interest, and EG-VEGF (PK1) research was limited by the availability of the

antibodies and reagents. Little is also known about this research protocol and therefore it can't be regarded as relevant. Furthermore the authors didn't take the myometrium into consideration.

In the first trimester it is difficult to prognose which pregnancy is likely to develop the PE. Therefore establishing the difference in PK1 mRNA expression between second and third trimester is essential to assess its influence on PE etiology. As stated in our research the PK1 production is significantly reduced in PE placentas. Hoffmann *et al.* (2008) results are therefore in contrast with our data. Authors obtained higher levels of the PK1 in the maternal serum of the patients with PE in the second and third trimester. Based on that we can't conclude whether the elevated concentration is the result of the greater trophoblast production as PK1 is being produced also in other places such as GI tract, ovaries and adrenals. Our data strongly suggest that placenta is not responsible for PK1 high levels in PE patients. Therefore, further research are necessary to compare in PE patients mRNA PK1 expression (in all places where PK1 is being produced) with plasma PK1 concentrations.

It needs to be highlighted that our paper is probably the first exploring mRNA PK1 and PKR1 expression in the myometrium of the healthy pregnant women and women with pregnancies complicated by the PE, even though no statistically significant differences between groups have been detected. Based on that we can conclude that pathology that lies at the background of the PE do not change the PK1 and PKR1 expression in the myometrium although it's much weaker than in placenta. Furthermore, we can conclude that myometrium as a target organ for the improper invasion of the trophoblast is not taking part in it actively.

Intracellur signaling is the inner activity of the cell in response to a ligand. In our research we received the statistically significant difference between normals and PE patients only in myometrium, after 30 minutes of the ligand (EG-VEGF) stimulation. In spite, the rise in pERK/tERK ratio was observed after 20 and 30 minutes in both placenta and myometrium of the controls, whereas in PE patients no rise and even falls in the intracellular signaling were observed, even though it didn't reach the statistical significance. In the controls, as was assessed using Spearman test, the pERK/tERK ratio in both the myometrium and the placenta was rising in time, whereas in PE patients it did not rise at all.

There are few remarks in the literature concerning PK1 presence in placenta (Hoffmann 2006), no paper focuses on PK1 presence in myometrium. One report concerns the influence of the PK1 on the MMP-2 and MMP-9 production by the villi (Hoffmann 2008). However, there are no studies assessing biological influence of the PK1 on the cell activity. The results obtained in our research, although they didn't reach statistical significance confirm the cell signaling in the placenta and in the myometrium of the PE patients being impaired. This is probably due to the PKR1 malfunctioning, as there is no difference in the number of the PKR1 between the groups. Whether the impaired functioning is the consequence of the structural changes of the PKR1, or the improper transformation in response to the ligand remains unclear and needs to be elucidated. In the light of the recent research (LeCouter *et al.* 2001; Hoffmann *et al.* 2006) and especially Huppertz data (2008), we can conclude, that changes in the PK1 expression or signaling may be the reason of the PE, especially this of the late origin.

In our study we were analyzing the immunolocalization of the PK1 and PKR1 in the placenta and the myometrium. There was no qualitative difference in the localization of the examined ligand nor its receptor between patients with PE and healthy controls. Confronting our results with those of Hoffmann *et al.* (2006) the conclusion appears to be obvious. Strong expression of the PK1 in syncytiotrophoblast (ST) may suggest that its expression may be dependent on its differentiation and further from its invasion. That would support our results stating the statistically significant greater presence of PK1 in placenta of the normals. Denison *et al.* (2008) showed the presence of the PK1 also in CT and fetal endothelium. Another paper (Hoffmann *et al.* 2006) suggests the presence of the PK1 in ST to be the highest between $8^{th}$ and $10^{th}$ week of the gestation, which may suggest that PK1 plays important role in critical, hypoxic time of the placentation. Immunolocalization of the PKR1 is similar in the placenta as this of the PK1, but different than PKR2 (Hoffmann 2007). Hoffmann *et al.* (2008) shows the presence of the PKR1 only in CT, weak in ST and EVT. As for PKR2, its presence was confirmed (Hoffmann 2008) only in ST, EVT, fetal endothelium and Hofbauer cells. As shown above, the data concerning PK1 receptors are incoherent. The presence of the PKR1 in Hofbauer cells may suggest the role of the PK1/PKR1 complex as a paracrine immunological modulator in placental macrophages.

The immunolocalization of the PK1 or PKR1 was never explored in the myometrium. The PK1 and PKR1 expression in the smooth muscle cells of the myometrium is generally weak and sometimes negative. The real question, however, is different. The prokineticins were originally described as a new factors mediating strong and constant contraction of the guinea pig intestine (Li 2001). Recently, new data (Hoogerwerf 2006) brought controversial results, suggesting that PK1 causes NO mediated dilatation of the proximal part of the murine colon. It may suggests that specific cellular environment may determine various receptor feedback thus expressing different phenotypes. Therefore it is worth explaining whether disturbances in PK1 production in syncytiotrophoblast or PKR1 impairement may affect human myometrium contractility (and cause the rise in systemic resistance in spiral arteries) during pregnancy.

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