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PhD thesis summary

Pregnancy proteins – molecular biological and biochemical analysis

Mgr. Alexandra Muravská

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Section:	**Biochemistry and pathobiochemistry**
Section chairman:	**Prof. MUDr. Jiří Kraml, DrSc.**
Workplace:	**Institute of Medical Biochemistry and Laboratory Diagnostics**
	1st Faculty of Medicine, Charles University in Prague and General University Hospital in Prague
	U nemocnice 2, Praha 2, 128 08
Supervisor:	**Prof. MUDr. Marta Kalousová, PhD.**
Supervisor - consultant:	**RNDr. Marie Jáchymová, PhD.**

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Contents

Abstract

The aim of this thesis was to establish methods for selected *PAPP-A* (*Pregnancy-Associated Plasma Protein A*) gene polymorphisms analysis and to study genetic background of PAPP-A and biochemical background of PAPP-A and P*l*GF (*Placental Growth Factor*) in relation to risk pregnancy. Secondly, the aim was to establish method for two-dimensional (2D) electrophoresis of amniotic fluid.

Methods for analysis of ten *PAPP-A* gene polymorphisms were established. These polymorphisms, PAPP-A and P*l*GF levels were studied in together 165 women in third trimester pregnancies complicated with threatening preterm labor (n=98), preeclampsia (n=35), IUGR (*Intrauterine Growth Restriction*) (n=34) and ICP (*Intrahepatic Cholestasis of Pregnancy*) (n=15). 114 healthy pregnant women served as controls. The method for 2D electrophoresis of amniotic fluid was established.

Preeclamptic patients had significantly higher frequency of TT genotype of Cys327Cys (C/T) *PAPP-A* gene polymorphism compared to controls. Patients with ICP had increased serum levels of PAPP-A compared to controls, in patients with threatening preterm labor PAPP-A levels were rather decreased. P*l*GF levels did not differ from control group in patients with ICP and threatening preterm labor. Positive correlation was found between PAPP-A and P*l*GF in group of healthy pregnant controls. Negative relationship was found between P*l*GF and erythrocytes and hemoglobin and between P*l*GF and creatinine in patients with threatening preterm labor and IUGR, respectively.

Our results contribute to better understanding of the pathological mechanisms in risk pregnancies and can help to more effectively identify high-risk patients to provide early and appropriate care. However, further study with larger groups of patients with risk pregnancies is needed to confirm our results.

Abstrakt

Cílem této disertační práce bylo zavést metodiky analýzy vybraných polymorfizmů genu *PAPP-A* (*Pregnancy-Associated Plasma Protein A*) a studovat genetické pozadí genu *PAPP-A* a sérové koncentrace PAPP-A a P*l*GF (*Placental Growth Factor*) ve vztahu k patologickému těhotenství. Dalším cílem bylo zavést metodiku dvourozměrné (2D) elektroforézy plodové vody.

Byla zavedena metoda na analýzu deseti polymorfizmů genu *PAPP-A*. Tyto polymorfizmy, sérové koncentrace PAPP-A a plasmatické koncentrace P*l*GF byly studovány u celkově 165 těhotných pacientek ve třetím trimestru gravidity s hrozícím předčasným porodem (N=98), preeklampsií (N=35), růstovou retardací plodu IUGR (N=34) a benigní těhotenskou cholestázou (N=15). 114 zdravých těhotných žen sloužilo jako kontroly. V rámci této práce byla zavedena 2D elektroforéza plodové vody.

U pacientek s preeklampsií jsme nalezli signifikantně častější výskyt genotypu TT polymorfizmu Cys327Cys (C/T) genu *PAPP-A* v porovnání s kontrolami. Pacientky s těhotenskou cholestázou vykazovaly trend k zvýšeným hladinám PAPP-A, kdežto u pacientek s hrozícím předčasným porodem byly tyto hodnoty spíše nižší. U pacientek s těhotenskou cholestázou a hrozícím předčasným porodem se hladiny P*l*GF nelišily od kontrolní skupiny. Pozitivní korelace P*l*GF s hladinami PAPP-A byla nalezena u skupiny zdravých těhotných kontrol. U pacientek s hrozícím předčasným porodem byl nalezen negativní vztah P*l*GF s hladinami erytrocytů a hemoglobinu v oběhu matky. U pacientek s IUGR byla nalezena negativní korelace P*l*GF s kreatininem.

Naše výsledky přispívají k pochopení patologických mechanizmů komplikovaných těhotenství a mohou pomoci k efektivnější identifikaci rizikových pacientek s cílem včas zajistit vhodnou péči. Nicméně, pro potvrzení našich výsledků je potřeba další studium s větším počtem pacientek s patologickým těhotenstvím.

1. Introduction

Pregnancy is one of the most important physiological processes of human life. At the beginning of the pregnancy a fetoplacental unit is formed in the uterus of the mother, where the communication between mother and fetal blood circulation takes place. Proteins produced by the fetoplacental unit are important for the physiological development of the fetus and are detectable mainly in the maternal circulation and amniotic fluid. Some of these proteins are important markers commonly used for screening of congenital genetic defects of fetus. The latest research indicates that pregnancy proteins can be useful markers of adverse pregnancy outcomes and other diseases unrelated to pregnancy.

Pregnancy-Associated Plasma Protein A (PAPP-A) is a glycoprotein belonging to the metzincin family of metalloproteinases. It was first described in 1974 [1] as one of the four proteins of placental origin. PAPP-A exists in pregnancy serum as a heterotetrameric 2:2 complex with the proform of eosinol Major Basic Protein (proMBP) [2]. This protein is also expressed in tissues of nonpregnant women and men in concentrations 100-1000 times lower than in 1st trimester of pregnancy. PAPP-A is routinely used for screening of Down syndrome in the 1st trimester of pregnancy [3], is elevated in preeclampsia [4] and more and more evidences indicates its potential use in other diseases diagnostics [5,6].

The human PAPP-A gene is located on chromosome 9q33.1 [7], spans over 200 kilobases of DNA and contains 22 exons ranging in length from 72 to 1063 nucleotides [8].

Placental Growth Factor PlGF belonging to the Vascular Endothelial Growth Factor (VEGF) family, which applies in the development and growth of vascular and lymphatic endothelium, particularly during embryogenesis [9]. PlGF was first described in 1991 [10] and 10 years later its three-dimensional structure was elucidated [11]. PlGF is primarily expressed by placental trophoblasts during pregnancy [12].

The human PlGF gene is located on chromosome 14 and includes together seven exons [13].

Proteomic analysis of amniotic fluid has great potential in the field of pathological pregnancies diagnostics. Amniotic fluid is appropriate medium to study both pregnancy and fetus specific proteins and can help improve the understanding of the physiology of pregnancy and fetus development.

2. Aims of the study

1. To establish the methods for the single nucleotide polymorphisms (SNP) detection:

- To establish the method for detection of SNP Pro324Leu (rs445154), Pro325Leu (rs445159) a Cys327Cys (rs12375498) in exon 2 of the *PAPP-A* gene.

- To establish the method for detection of SNP Arg654Lys (rs432500), Ala678Pro (rs34371232) a Thr686Ala (rs35578777) in exon 5 of the *PAPP-A* gene.

- To establish the method for detection of SNP Glu715Glu (rs35407884) in exon 6 and C/G (rs13290387) in intron 6 of the *PAPP-A* gene.

- To establish the method for detection of SNP Phe802Leu (rs1063409), Ser827Ser/Leu (rs34087604) in exon 7 of the *PAPP-A* gene.

2. Clinical application of the established methods:

- To analyze these SNPs in patients with risk pregnancies (threatening preterm labor, preeclampsia, intrauterine growth restriction, intrahepatic cholestasis of pregnancy) and in control group of healthy pregnant and nonpregnant women and to study its association with clinical characterization and laboratory parameters.

3. To determine serum concentrations of PAPP-A protein and plasma concentrations of P*l*GF in patients with risk pregnancies (threatening preterm labor, preeclampsia, intra-uterine growth restriction, intrahepatic cholestasis of pregnancy) and in control group of healthy pregnant women.

4. To establish the method for two-dimensional electrophoresis as a starting point for proteomic analysis of amniotic fluid.

3. Materials and methods

3.1 Parameters of clinical study

165 Caucasian women (mean age 30.8 ± 4.7 years) from Czech Republic were included in this single center study. All studied patients were in the third trimester of pregnancy.

98 women (mean age 30.7 ± 5.1 years) with symptoms of threatening preterm labor and 35 patients (mean age 31.3 ± 4.1 years) with hypertension in pregnancy or preeclampsia were enrolled in the study. 21 women had hypertension and proteinuria more than 300 mg per day. 14 women with hypertension had not proteinuria at the time of blood collection, but either they finally developed it during follow up or had increased serum levels of uric acid above 320μmol/l. 34 women (mean age 31.1 ± 4.6 years) had a fetus with intrauterine growth restriction and 15 women (mean age 31.7 ± 3.4 years) suffered from intrahepatic cholestasis of pregnancy.

114 healthy pregnant women in the third trimester of pregnancy (mean age 30.1± 4.3 years) were followed up during prenatal care in the Department of Obstetrics and Gynaecology of the General University Hospital in Prague and studied as controls. 48 healthy women (mean age 26.1± 4.2 years) served as non-pregnant controls.

The study was performed in accordance with principles of the Declaration of Helsinki and approved by the Institution Ethics Review Board. All patients gave their informed consent prior to entering the study.

3.2 Methods

Single nucleotide polymorphisms of the *PAPP-A* gene analysis

Isolation of DNA was performed by modified salting out procedure after Miller et al. [14] within the week of blood collection. DNA concentration was measured with the spectrophotometer ND-1000 (NanoDrop®, USA).

Selected single nucleotide polymorphisms of the *PAPP-A* gene were then analyzed by direct sequencing. First, the target DNA fragments with polymorphic sites were amplified by polymerase chain reaction (PCR) and then purified to remove non-specific DNA fragments, salts and reaction components either directly from the reaction mixture or by electrophoresis. Purified DNA fragments were further subjected to another PCR with addition of dideoxy phosphates with fluorescent dyes. Ethanol precipitation followed to remove salts and PCR reaction components.

Finally, samples were loaded into sequencing instrument CEQ 8000 (Beckman Coulter, USA) and nucleotide sequence of DNA fragments was determined.

Biochemical analysis

In whole group of patients and pregnant controls PAPP-A serum levels were assessed immunochemically, using the TRACE method (Time Resolved Amplified Cryptate Emission) Commercial kit KRYPTOR-PAPP-A (Brahms, Germany) was used. In the group of 110 patients and 41 healthy pregnant controls P*l*GF plasma levels were determined using ELISA (Enzyme-Linked ImmunoSorbent Assay) and commercial kit Quantikine (RD Systems, USA).

Routine biochemical parameters were assessed by commercially available kits using certified biochemical techniques with automated analyzers Modular (Roche Diagnostics, Germany) and Beckman Coulter LH750 Hematology analyzer (Beckman Coulter, USA).

2D electrophoresis of amniotic fluid

A two-dimensional electrophoresis proceeded in two major steps. First, denatured proteins were transferred to a gel strip with a non-linear pH gradient. During isoelectric focusing proteins became focused into sharp stationary bands with each protein positioned at a point in the pH gradient corresponding to its pI. Strip was then laid sideways across the top of a polyacrylamide gel allowing the proteins to be separated in the second dimension according to size in the presence of SDS. Finally, proteins were detected with Coomassie blue. Mass spectrometry can be used for identification of individual spots on the gel.

Statistical analysis

Results of biochemical parameters are expressed as mean±standard deviation (SD). Statistical analysis of group differences was performed by unpaired t-test and ANOVA (one-way analysis of variance) followed by post test analysis. The significances of the differences in the genotype and allelic distribution of the *PAPP-A* gene polymorphisms were tested using the χ^2 (chi-square) test. Association between parameters was determined by using Pearson´s coefficients. For statistical analyses, the software "Prism 5", GraphPad Software Inc, was used.

The results were considered as statistically significant at $p < 0.05$.

4. Results

Single nucleotide polymorphisms of the *PAPP-A* gene

I have established the methods for analysis of ten *PAPP-A* gene polymorphisms: Pro324Leu (rs445154), Pro325Leu (rs445159) and Cys327Cys (rs12375498) in exon 2; Arg654Lys (rs432500), Ala678Pro (rs34371232) and Thr686Ala (rs35578777) in exon 5; Glu715Glu (rs35407884) in exon 6; C/G (rs13290387) in intron 6; Phe802Leu (rs1063409), Ser827Ser/Leu (rs34087604) in exon 7.

Polymorphisms in exon 5 and 7 were analyzed in 103 patients with risk pregnancies, 52 healthy pregnant controls and 48 healthy non-pregnant controls. We have not found mutated allele in any of the studied subject and thus we have not carried out an analysis of these exons furthermore. Polymorphisms in exon 2, 6 and intron 6 of the *PAPP-A* gene were analyzed in 165 patients and 114 healthy pregnant women. Among our studied group, we have found only Cys327Cys (rs12375498) polymorphism in exon 2 and C/G (rs13290387) polymorphism in intron 6 (Table 1) [15, 16].

Table 1. Allele frequencies of the *PAPP-A* gene polymorphisms in patients with risk pregnancies.

	Number of patients	*Polymorphism*	*dbSNP number*	*Wild-type allele (frequency)*	*Mutated allele (frequency)*
Exon 2	165	Pro324Leu	rs445154	C (1.00)	T (0.00)
		Pro325Leu	rs445159	C (1.00)	T (0.00)
		Cys327Cys	rs12375498	C (0.74)	T (0.26)
Exon 5	103	Arg654Lys	rs432500	G (1.00)	A (0.00)
		Ala678Pro	rs34371232	G (1.00)	C (0.00)
		Thr686Ala	rs35578777	A (1.00)	G (0.00)
Exon 6	165	Glu715Glu	rs35407884	A (1.00)	G (0.00)
Intron 6	165	C/G	rs13290387	G (0.57)	C (0.43)
Exon 7	103	Phe802Leu	rs1063409	T (1.00)	C (0.00)
		Ser827Ser/Leu	rs34087604	TC (1.00)	C-/-- (0.00)

dbSNP – single nucleotide polymorphisms database of The National Center for Biotechnology Information (NCBI).

The allelic and genotype frequencies between the entire group of patients with risk pregnancies and healthy pregnant women did not differ both in Cys327Cys polymorphism (exon 2) and C/G polymorphism (rs13290387, intron 6) and they both were in Hardy-Weinberg

equilibrium. Concerning the Cys327Cys (C→T) polymorphism of the *PAPP-A* gene, patients with preeclampsia had significantly higher frequency of mutated TT genotype compared to controls (p<0.01). Moreover, this result was supported by the weaker but the same trend of T allele in patients with preeclampsia compared to controls (p=0.056) (Table 2) [16].

Table 2. Genotype and allelic frequencies of the Cys327Cys *PAPP-A* gene polymorphism in the subgroups of patients divided based on clinical characteristics and in the control group.

Cys327Cys (rs12375498) exon 2	Genotypes [%]				Alleles [%]		
	CC	CT	TT		C	T	
Preterm labor (N=98)	54	40	6		74	26	
Preeclampsia (N=35)	40	51.4	8.6	*	66	34	+
IUGR (N=34)	53	41	6		74	26	
ICP (N=15)	67	33	0		83	17	
Healthy pregnant controls (N=114)	61	35	4		78	22	

Comparison was performed between subgroups of patients with pathological pregnancy versus controls. χ^2 (chi-square) test was used, *p<0.01 vs. controls, +p=0.056 vs. controls.

Laboratory parameters

Patients with preeclampsia had significantly increased serum levels of PAPP-A compared to controls (Table 3). When we divided preeclamptic patients into subgroup of patients with proteinuria (N=21, PAPP-A: 128.7±83.7 IU/l) and subgroup of patients who developed proteinuria during follow up or had increased serum levels of uric acid above 320μmol/L (N=14, PAPP-A: 108.4±69.6 IU/l) and compared to control group (N=114, PAPP-A: 82.8±49.4 IU/l) of healthy pregnant women, we found even stronger difference in PAPP-A levels between subgroup of preeclamptic patients with proteinuria and control group (p<0.01, ANOVA p=0.0025). We did not find any statistical significant difference in PAPP-A levels between subgroup of patients without proteinuria and control group.

Group of patients with ICP showed increased levels of PAPP-A as well (p=0.01 vs. controls, t-test), whereas patients with preterm labor had a tendency (p=0.01 vs. controls, t-test) to lower levels of PAPP-A (Table 3).

Correlation analysis showed significant relationship between PAPP-A and CRP in the patients with IUGR (r=0.49, p= 0.007) [16].

Patients with preeclampsia and IUGR had significantly decreased P*l*GF plasma levels compared to controls (Table 3). When we divided preeclamptic patients into subgroup of patients with proteinuria (N=21, P*l*GF: 92.2±67.4 pg/ml) and subgroup of patients who developed proteinuria during follow up or had increased serum levels of uric acid above 320μmol/L (N=11, P*l*GF: 132.5±94.4 pg/ml) and compared to control group (N=41, P*l*GF: 224.7±152.6 pg/ml) of healthy pregnant women, we found even stronger difference in P*l*GF levels between subgroup of preeclamptic patients with proteinuria and control group (p<0.001, ANOVA p=0.0006). We did not find any statistical significant difference in P*l*GF levels between subgroup of patients without proteinuria and control group.

Correlation analysis showed significant positive relationship between P*l*GF and PAPP-A in healthy pregnant women. A negative relationship between P*l*GF and erythrocyte count and hemoglobin serum levels was found in patients with threatening preterm labor. In patients with IUGR, a negative correlation was found between P*l*GF and creatinine.

Table 3. PAPP-A and P*l*GF levels in patients with risk pregnancies and healthy pregnant women in third trimester of pregnancy.

	PAPP-A [IU/l]	*Number*	*PlGF [pg/ml]*	*Number)*
Preterm labor	65.15±44.27	98	218.6±170.47	35
Preeclampsia	120.92±78.18**	35	106.04±78.64**	32
IUGR	94.16±79.79	34	103.38±99.17**	30
ICP	118.35±67.23	15	197.65±138.35	13
Healthy pregnant controls	82.8±49.9	114	224.71±152.60	41
ANOVA (p)	<0,0001		<0,0001	

Results are means±standard deviation. One-way ANOVA was performed followed by Bonferroni's Multiple Comparison Test. **p<0.01, vs. controls.

2D electrophoresis of amniotic fluid

I have established the method for 2D electrophoresis as a starting point for proteomic analysis of amniotic fluid. The work on this method was done during my practical placement in the laboratory of professor Luciano Binaglia, at the Department of Internal Medicine, Section of Biochemistry, University of Perugia, Perugia, Italy.

The aim was to establish and to optimize the method for 2D electrophoresis of amniotic

fluid. Together 15 amniotic fluid samples of pregnant patients with unknown clinical characteristics were obtained for analysis.

Figure 1 depicts a 2D (two dimensional) protein spot map of the amniotic fluid sample. Black spots correspond to individual proteins of the amniotic fluid, which can be further identified by mass spectrometry. Big black spot at the top of the map represents the albumin, which is the most abundant protein of the amniotic fluid.
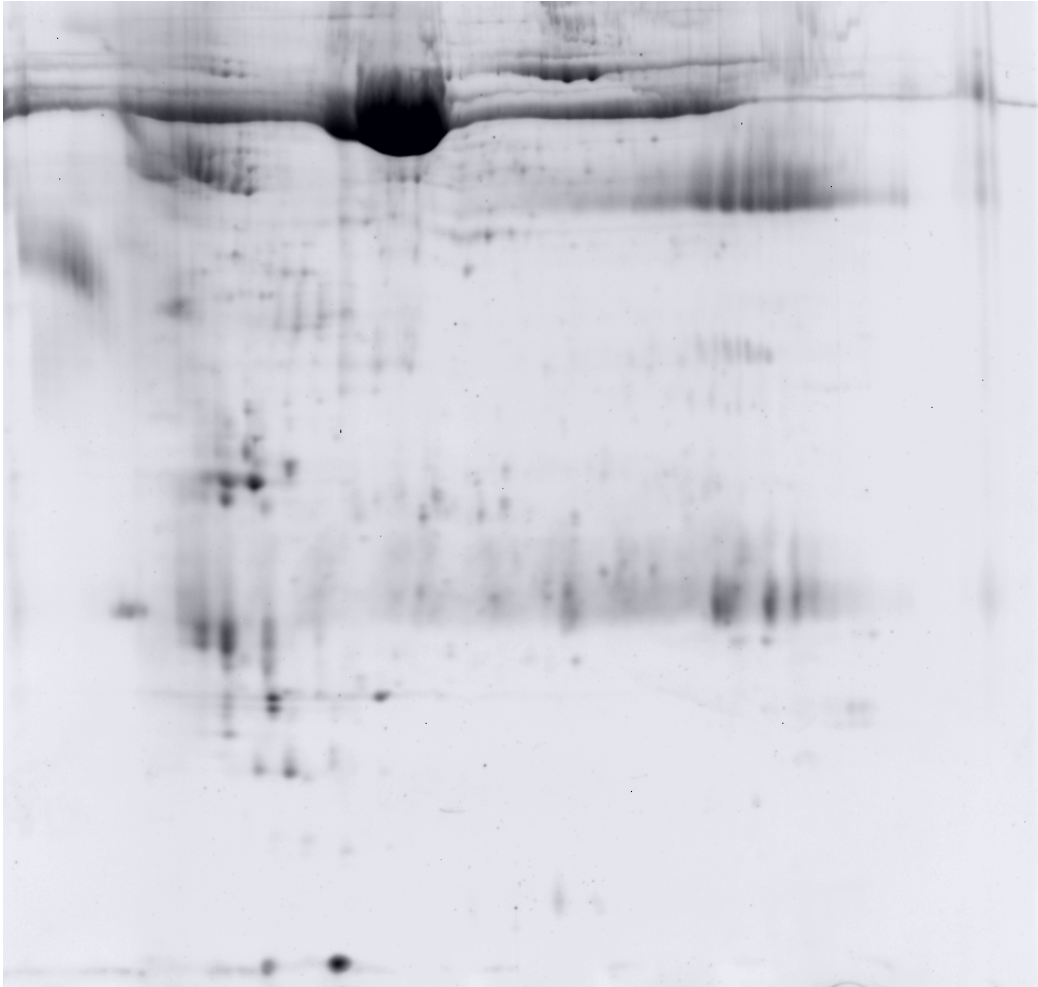
Figure 1. 2-D protein spot map of the amniotic fluid sample no.1.

5. Discussion

We have established for the first time the methods for analysis of ten *PAPP-A* gene polymorphisms localized in exon 2, 5, 6, 7 and intron 6 based on direct sequencing of the genomic DNA - Pro324Leu (rs445154), Pro325Leu (rs445159) and Cys327Cys (rs12375498) in exon 2; Arg654Lys (rs432500), Ala678Pro (rs34371232) and Thr686Ala (rs35578777) in exon 5; Glu715Glu (rs35407884) in exon 6; C/G (rs13290387) in intron 6; Phe802Leu (rs1063409), Ser827Ser/Leu (rs34087604) in exon 7.

Polymorphisms in exon 5 and 7 were analyzed in 103 patients with risk pregnancies, 52 healthy pregnant controls and 48 healthy non-pregnant controls. We have not found mutated allele in any of the studied subject [15] and thus we have not carried out an analysis of these polymorphisms furthermore. Polymorphisms in exon 2, 6 and intron 6 of the *PAPP-A* gene were analyzed in 165 patients and 114 healthy pregnant women [16]. Among our studied group, we have found only Cys327Cys (rs12375498) polymorphism in exon 2 and C/G (rs13290387) polymorphism in intron 6.

The allelic and genotype frequencies between the entire group of patients with risk pregnancies and healthy pregnant women did not differ both in Cys327Cys polymorphism and C/G polymorphism (rs13290387) and they both were in Hardy-Weinberg equilibrium, which proves that studied groups were appropriately selected.

Our results indicate for the first time the connection of mutated TT genotype of Cys327Cys polymorphism of the *PAPP-A* gene with preeclampsia ($p < 0.01$). This result was supported by the weaker but the same trend of T allele in patients with preeclampsia compared to controls ($p = 0.056$). However, these results can be affected by the relatively small group of patients with preeclampsia (N=35).

Since yet, no study focused on Cys327Cys polymorphism of the *PAPP-A* gene in patients with preeclampsia has been published. On the other hand, many studies have revealed the association of particular gene to preeclampsia [17]. We did not find any association of C/G (rs13290387) polymorphism with preterm labor, preeclampsia, IURG or ICP. Nevertheless, this polymorphism was found to be connected to an increased risk of acute myocardial infarction [18] and recurrent pregnancy loss [19].

Our study has shown significantly increased levels of PAPP-A in patients with preeclampsia in third trimester compared to healthy pregnant controls. Previous studies have already shown similarly to our study elevated serum levels of PAPP-A in the last month of gestation in preeclamptic pregnancies [4, 20]. On the other hand, Bersinger and Ødegård [21] did

not find any significant difference in PAPP-A serum levels between the patients with preeclampsia and healthy pregnant controls at 25th and 33rd week of pregnancy. Moreover, Bersinger et al. reported the same results in pregnancies complicated with IUGR [21], which is in accordance with our results. In our study, patients with preterm labor had a tendency to lower levels of PAPP-A (p=0.01 vs. controls, t-test) in 3rd trimester, although ANOVA analysis was not significant. In a study from 1984, PAPP-A levels did not changed in the 2nd and 3rd trimester in patients with preterm labor compared to corresponding values in normal pregnancy [22].

We demonstrate for the first time elevated PAPP-A serum levels in patients with ICP compared to healthy pregnant controls (p=0.01, t-test) in third trimester of pregnancy. The difference between the groups can be considered as weak, since the ANOVA analysis was not significant, however the unpaired t-test showed a strong significance when concerning only these two groups. Another limitation is a relatively small number of patients with ICP (N=15).

We observed a positive correlation between serum PAPP-A and CRP among patients with IUGR (r=0.49, p= 0.007). The same positive correlation has been observed in patients with renal failure or after renal transplantation [23, 24].

Preeclampsia and IUGR are pathological states closely related to placental dysfunction, which affect the levels of P*l*GF in maternal blood circulation. More studies have confirmed reduced levels of P*l*GF in patients with preeclampsia throughout gestation [21, 25]. Preeclamptic patients with IUGR fetuses had even lower P*l*GF levels than patients with preeclampsia and without IUGR [26]. Our study confirmed decreased P*l*GF levels in patients with preeclampsia in the 3rd trimester of pregnancy. We have observed the same results in the group of patients with IUGR as well. Reduced P*l*GF levels have been described in patients with IUGR caused by placental dysfunction after 20th week of gestation and in both maternal serum and serum from the umbilical vein at the time of delivery [27, 28].

Placental dysfunction may play an important role in the pathophysiology of spontaneous preterm labor, with P*l*GF levels changes detectable close to the onset of spontaneous preterm labor and delivery [29, 30]. P*l*GF levels in our group of patients with threatening preterm labor did not differ from the values of the control group, although all patients gave birth within 24 hours after blood collection for biochemical analysis.

Correlation analysis showed significant positive relationship between P*l*GF and PAPP-A in healthy women in the third trimester of pregnancy. These results are complementary to a study that confirmed a positive correlation between P*l*GF and PAPP-A in healthy women in the 1st trimester of pregnancy [31, 32]. In patients with IUGR, a negative correlation was found

between P*l*GF and creatinine. The same negative correlation was found in patients with preeclampsia [26], although our group with preeclampsia did not confirm these results.

A negative relationship between P*l*GF and erythrocyte count and hemoglobin serum levels was found in patients with threatening preterm labor. The same negative correlation was observed in patients with sickle cell anemia who developed pulmonary hypertension [33]. The study suggests the significance of hemolysis in the development of hypertension in patients with sickle cell anemia. Explanation of the relationship between erythrocyte count and hemoglobin serum levels and premature labor requires further study.

The results of this dissertation contribute to better understanding of pathological mechanisms in risk pregnancies and can help to identify high-risk patients more effectively to provide early and appropriate care. Proteomic analysis of amniotic fluid may help to improve prenatal diagnostics or current screening methods. It is possible that the discovery of specific markers in amniotic fluid will help to improve current diagnostic methods in maternal serum.

6. Conclusions

- I have established the methods for analysis of ten *PAPP-A* gene polymorphisms - Pro324Leu, Pro325Leu, Cys327Cys in exon 2; Arg654Lys, Ala678Pro, Thr686Ala in exon 5; Glu715Glu in exon 6 and C/G (rs13290387) in intron 6; Phe802Leu and Ser827Ser/Leu in exon 7 – based on direct sequencing of genomic DNA.

- Among our studied groups of 114 healthy controls and 165 patients with risk pregnancies (threatening preterm labor, preeclampsia, intrauterine growth restriction, intrahepatic cholestasis of pregnancy), we have found only Cys327Cys (C/T, rs12375498) polymorphism in exon 2 (allele C frequency 0.74) and C/G (rs13290387) polymorphism in intron 6 (allele G frequency 0.57). Polymorphisms in exon 5 and 7 were analyzed in smaller groups of patients and controls and among these individuals they were not found.

- Patients with preeclampsia had higher frequency of T allele and significantly higher frequency of TT genotype of the Cys327Cys *PAPP-A* gene polymorphism compared to controls.

- In whole group of patients and pregnant controls PAPP-A serum levels were assessed using the TRACE method. In the group of 110 patients and 41 healthy pregnant controls P*l*GF plasma levels were determined using ELISA.

- Patients with preeclampsia in the 3rd trimester of pregnancy had significantly increased serum levels of PAPP-A compared to controls. Group of patients with ICP showed increased levels of PAPP-A as well, whereas patients with preterm labor had a tendency to lower levels of PAPP-A.

- Patients with preeclampsia and IUGR had significantly decreased P*l*GF plasma levels compared to controls, whereas P*l*GF levels in patients with threatening preterm labor and ICP did not differ from control group.

- Correlation analysis showed significant positive relationship between P*l*GF and PAPP-A in healthy pregnant women. A negative relationship between P*l*GF and erythrocyte count and hemoglobin serum levels was found in patients with threatening preterm labor. In patients with IUGR, a negative correlation was found between P*l*GF and creatinine.

- I have established the method for 2D electrophoresis of amniotic fluid.

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List of original articles

1. Publications *in extenso* related to the thesis

a) with IF

1. <u>Germanová A</u>, Jáchymová M, Germanová A, Koucký M, Hájek Z, Zima T, Kalousová M. Pregnancy associated plasma protein-A polymorphisms in patients with risk pregnancies. Folia Biologica 2011; 57: 82-85. **IF = 0.729**

2. <u>Muravská A</u>, Germanová A, Jáchymová M, Hájek Z, Švarcová J, Zima T, Kalousová M. Association of Pregnancy-associated plasma protein A polymorphism with preeclampsia – A pilot study. Clin Biochem 2011; 44: 1380-1384. **IF = 2.043**

2. Publications *in extenso* with different objectives

a) with IF

1. Kalousová M, <u>Germanová A</u>, Jáchymová M, Mestek O, Tesař V, Zima T. A419C (E111A) polymorphism of the glyoxalase I gene is associated with vascular complications in chronic hemodialysis patients. Ann N Y Acad Sci 2008; 1126: 268-271. **IF = 2.303**

2. <u>Germanová A</u>, Germanová A, Tesařová P, Jáchymová M, Zima T, Kalousová M. Glyoxalase I Glu111Ala polymorphism in patients with breast cancer. Cancer Invest 2009; 27(6): 655-660 **IF = 2.105**

3. Kalousová M, Jáchymová M, <u>Germanová A</u>, Kuběna AA, Tesař V, Zima T. Genetic predisposition to advanced glycation end products toxicity is related to prognosis of chronic hemodialysis patients. Kidney Blood Press Res 2010; 33(1): 30-36. **IF = 1.500**

4. Škrha jr. J, Kalousová M, Švarcová J, <u>Muravská A</u>, Kvasnička J, Landová L, Zima T, Škrha J. Relationship of soluble RAGE and RAGE ligands HMGB1 and EN-RAGE to endothelial dysfunction in Type 1 and Type 2 diabetes mellitus. Exp Clin Endocr Diab 2011, *in press*. **IF = 1.826**

5. Jáchymová M, <u>Muravská A</u>, Paleček T, Kuchyňka P, Řeháková H, Magage S, Král A, Zima T, Horký K, Linhart A. Genetic variation screening of TNNT2 gene in a cohort of patients with hypertrophic and dilated cardiomyopathy. Physiol Res 2011, *in press*.   **IF = 1.646**

b) without IF

1. Jáchymová M, Brabcová I, <u>Germanová A,</u> Slatinská J, Mestek O, Matl I, Zima T, Viklický O, Kalousová M. A419C polymorphism of glyoxalase I gene, renal function and histological finding at 12 months after renal transplantation. Int J Nephrol Urol 2010; 2(4): 504-513.