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Genetic backround and new biochemical markers in pathological pregnancy

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ABSTRACT

Receptor for advanced glycation end products (RAGE), its soluble form (sRAGE) and glyoxalase 1 (GLO 1) are important part of pathogenesis of many chronic diseases.

The aim of this thesis was to elucidate role of sRAGE, four chosen *RAGE* polymorphisms and one *GLO 1* polymorphism in physiologic pregnancy and in pregnancy with complications.

Serum sRAGE levels were determined in healthy pregnant women (N=120) and in pregnancies complicated with threatening preterm labor (N=99), preeclampsia (N=35), intrauterine growth restriction (IUGR) (N=22) and intrahepatic cholestasis of pregnancy (ICP) (N=14). Four *RAGE* polymorphisms (*RAGE* -429T/C, -374A/T, *RAGE* Gly82Ser (557G/A), *RAGE* 2184A/G) and one *glyoxalase 1* polymorphism *GLO 1* Glu111Ala (419A/C) were studied in the same population of healthy pregnant women and women with pathological pregnancy.

Serum sRAGE levels are low in comparison to non-pregnant controls, but they vary during the physiologic pregnancy. Serum sRAGE levels are low in the 1st trimester, increased in the 2nd trimester and again decreased in the 3rd trimester. Women with premature labor have significantly decreased serum sRAGE levels in comparison to women with threatening premature labor and in comparison to healthy pregnant women. sRAGE correlates negatively with leukocyte count in preterm labor. Patients with preeclampsia have significantly increased serum sRAGE levels compare to healthy pregnant controls. sRAGE correlates positively with proteinuria, with serum uric acid and creatinine level. Serum sRAGE levels are not affected in patients with IUGR or ICP. sRAGE correlates positively with serum uric acid and creatinine level in patients with IUGR. sRAGE correlates negatively with serum alanine amino transferase (ALT) level in patients with ICP. There are no differences in genotype or allelic frequencies of studied RAGE and glyoxalase 1 polymorphisms among studied groups.

These results partly elucidate pathogenesis of pathological pregnancies. It might help to uncover high risk patients and provide them early adequate prenatal care. Further studies with larger studied group (especially patients with preeclampsia, IUGR, ICP) are still needed to confirm results.

ABSTRAKT

Receptor pro produkty pokročilé glykace (RAGE), jeho solubilní forma (sRAGE) a glyoxalasa 1 jsou důležitou součástí patogeneze mnoha chronických nemocí.

Cílem dizertační práce bylo objasnit význam sRAGE, čtyř polymorfizmů genu pro *RAGE* a jednoho polymorfizmu genu *glyoxalasy 1* ve fyziologickém i patologickém těhotenství.

Koncentrace sRAGE v séru byla stanovena u zdravých těhotných žen (N=120), u žen s hrozícím předčasným porodem (N=99), s preeklampsií (N=35), s růstovou retardací plodu (IUGR) (N=22) a s těhotenskou cholestázou (ICP) (N=14). Ve stejné skupině zdravých těhotných žen a žen s patologickým těhotenstvím byly stanoveny čtyři *RAGE* polymorfizmy (*RAGE* -429T/C, -374A/T, *RAGE* Gly82Ser (557G/A), *RAGE* 2184A/G) a jeden polymorfizmus glyoxalasy 1 *GLO 1* Glu111Ala (419A/C).

Hladina sRAGE v séru zdravých těhotných žen byla signifikantně nižší v porovnání s netěhotnými kontrolami. Hladina sRAGE se v průběhu těhotenství měnila, byla nízká v 1.trimestru, stoupala v 2.trimestru a zase klesala ve 3.trimestru a před porodem.

Těhotné pacientky s předčasným porodem měly významně nižší sérové koncentrace sRAGE v porovnání s pacientkami s hrozícím předčasným porodem či v porovnání se zdravými kontrolami. Hladina sRAGE negativně korelovala s hladinou leukocytů u předčasného porodu. Pacientky s preeklampsií měly signifikantně vyšší sérovou koncentraci sRAGE ve srovnání se zdravými těhotnými ženami. Hladina sRAGE pozitivně korelovala s proteinurií, se sérovou hladinou kyseliny močové a kreatininu. Sérové koncentrace sRAGE nebyly změněny u pacientek s IUGR či ICP. Hladina sRAGE pozitivně korelovala se sérovou koncentrací kyseliny močové a kreatininu u pacientek s IUGR. Hladina sRAGE rovněž pozitivně korelovala se sérovou koncentrací alaninaminotransferázy (ALT) u pacientek s ICP. U sledovaných skupin nebyly nalezeny žádné rozdíly v genotypových či alelických frekvencích studovaných polymorfizmů genu pro *RAGE* a genu *glyoxalasy 1*.

Tyto výsledky částečně přispívají k pochopení patogeneze některých patologických stavů v těhotenství, což může být důležité při odhalování rizikových pacientek a zajištění adekvátní prenatální péče. Pro potvrzení našich výsledků je však nezbytné provést další studie s větším počtem subjektů, zejména pacientek s preeklampsií, IUGR a ICP.

1. Introduction

Pregnancy is an important part of human life. The development of an embryo in the uterus and its interaction with the mother's body is a complex process. Physiological pregnancy is associated with enhanced oxidative stress, and its importance in pathological pregnancy is considerably higher.

Receptor for advanced glycation end products (RAGE), its soluble form (sRAGE) and glyoxalase 1 are important in the pathogenesis of chronic diseases associated with altered oxidative status and microinflammation.

RAGE is a transmembrane receptor, member of immunoglobuline superfamily. It was first described by Neeper et al. in 1992 (Neeper M. et al., 1992) as a receptor able to bind advanced glycation end products (AGEs). Latter, other RAGE ligands were identified, such as S100 proteins, High Mobility Group protein 1, Amyloid β peptide. RAGE – ligand interaction results in activation of intracellular signaling pathways and thus activation of nuclear factor κB. Stimulation of RAGE causes generation of oxidative stress and triggering of inflammatory and proliferative processes (Schmidt AM. et al., 1995).

sRAGE is truncated form of RAGE, lacking transmembrane domain. sRAGE has two variants: endogenous soluble RAGE, secreted from cells and cleaved RAGE, produced by proteolytic cleavage from cell surface by membrane sheddases and matrix metalloproteinases. sRAGE acts as a decoy by binding RAGE ligands and thus, diminishes pathological effects mediated by RAGE (Hudson BI. et al., 2005).

The human *RAGE* gene is located on chromosome 6. It consists of 11 exons. Most of *RAGE* polymorphisms are very rare in population, so only few of them came to the center of attention. *RAGE -429T/C and -374T/A* polymorphisms, located in the gene promoter, effect transcriptional activity. *RAGE Gly82Ser (557G/A)* polymorphism in exon 3 is important for RAGE - ligand binding. *RAGE 2184A/G* polymorphism in intron 8 probably influences production of sRAGE.

Glyoxalase 1 is part of the glyoxalase system, which is responsible for the detoxification of AGEs precursors. It is a zinc metalloenzyme, which mostly metabolizes glyoxal and methylglyoxal. Decreased glyoxalase 1 activity leads to accelerated formation of AGEs. Glyoxalase 1 activity is also decreased by RAGE - S100A12 interaction (Thornalley PJ., 2007). *Glyoxalase 1* gene is located on chromosome 6 and comprises 6 exons. The most studied polymorphism located in exon 4, *Glu111Ala (419A/C),* is also associated with reduced activity of glyoxalase 1 (Barua M. et al., 2011).

2. Aim of the Study

The importance of receptor for advanced glycation end products, soluble receptor for advanced glycation end products and glyoxalase 1 has been proven in the pathogenesis of chronic diseases such as inflammatory diseases, diabetes mellitus, cardiovascular diseases, chronic renal diseases and cancer. Common sign of these diseases is altered oxidative status and microinflammation. Oxidative stress and microinflammation is also typical for physiologic pregnancy and even more for pregnancy complication. The aim of the work was to explore role of RAGE, sRAGE and glyoxalase 1 in physiologic and pathologic pregnancy.

1. Assessment of sRAGE serum levels

- To study sRAGE serum levels and their dynamics during physiologic pregnancy.
- To study sRAGE serum levels in pregnant women with threatening preterm labor and compare them with women with physiologic pregnancy.
- To study sRAGE serum levels in pregnant women with other pregnancy induced diseases and compare them with women with physiologic pregnancy.

2. Assessment of RAGE polymorphisms - rs1800625 RAGE -429 T/C, rs1800624 RAGE -374 A/T, rs2070600 RAGEGly82Ser (557 G/A), rs3134940 RAGE 2184 A/G and Glyoxalase 1 polymorphism - rs4746 GLO 1 Glu111Ala (419A/C)

- To study these SNPs in pregnant women with threatening preterm labor.
- To study these SNPs in pregnant women with other pregnancy induced diseases.
- To study these SNPs in healthy pregnant controls for comparison.

3. Materials and methods

3.1 Study population

120 Caucasian healthy pregnant women (mean age 30±4 years) were enrolled in the study. At the time of blood collection, 27 women were in the 1st trimester, 25 women were in the 2nd trimester and 68 women were in the 3rd trimester. All of them delivered at term.

99 pregnant patients (mean age 31±5 years) with threatening preterm labor were enrolled in the study. The mean week of delivery was 32±5 weeks. 75 patients delivered within 24 hours after enrollment in the study.

35 pregnant patients (mean age 31±4 years) suffering from preeclampsia were enrolled in the study. All patients had hypertension and proteinuria above 300 mg per 24 hours or developed such proteinuria during the follow-up. The mean week of labor was 37±4 weeks.

22 pregnant patients (mean age 29±4 years) with intrauterine growth restriction (IUGR) were enrolled in the study. IUGR was diagnosed as retardation of fetal growth under the 3rd percentile of regional growth curves. The mean week of delivery was 35±4 weeks.

14 pregnant patients (mean age 32±4 years) with diagnosis of intrahepatic cholestasis of pregnancy (ICP) were included in the study. The mean week of labor was 38±2 weeks.

24 non-pregnant healthy women (mean age 28±5 years) served as controls.

The study was performed in accordance with the principles of the Declaration of Helsinki and approved by the local Ethical Committee. All patients gave their informed consent prior to entering the study.

3.2 Methods

sRAGE assessment

sRAGE was assessed with enzyme linked immunosorbent assay (ELISA). Kits (Quantikine, RD Systems, Minneapolis, MN, USA) were used according to the protocol of the manufacturer. The plate is coated with monoclonal antibodies against sRAGE and polyclonal antibodies are used for detection.

RAGE and glyoxalase 1 polymorphism assessment

Modified salting-out method was used for DNA extraction (Miller SA. et al., 1988). For further genetic analysis PCR - RFLP (polymerase chain reaction - restriction fragment length polymorphism analysis) was used. Primers described in Table 3.1 were used for amplification of DNA. PCR was conducted in a 25µl volume containing 100 ng of genomic

DNA and 5 µM of each primer. Annealing temperature was 59.5 °C (60 °C for *RAGE* 2184A/G and *Glyoxalase 1* Glu111Ala polymorphism). Final extension occurred at 72 °C for 7 minutes. Restriction analysis was performed with all PCR products using restriction enzymes, *AluI* for *RAGE -429T/C* and *RAGE Gly82Ser*, *MfeI* for *RAGE -374T/A, BsmFI* for *RAGE 2184A/G* and *BsmAI* for *GLO1 Glu111Ala* for overnight at 37 °C (65 °C for *BsmFI*). The restriction products were separated by electrophoresis in 3% agarose gel and visualized in UV light after ethidium bromide staining. Restriction products of particular *RAGE* a *Glyoxalase 1* polymorphism are shown in Figure 3.1 and 3.2

Table 3.1 Primers used for the amplification of studied polymorphism

Polymorphism	5' ...sequence... 3'	Product size
RAGE -429T/C	sense: GGG GCA GTT CTC TCC TCA CT antisense: GGT TCA GGC CAG ACT GTT GT	250 bp
RAGE -374T/A	sense: GGG GCA GTT CTC TCC TCA CT antisense: GGT TCA GGC CAG ACT GTT GT	250 bp
RAGE Gly82Ser	sense: GTA AGC GGG GCT CCT GTT GCA antisense: GGC CAA GGC TGG GGT TGA AGG	397 bp
RAGE 2184A/G	sense: GGCCTCAGGACCAGGGAACCTACA antisense: TTGGTCAGGCTGGTCTCGAACTCC	402 bp
GLO1 Glu111Ala	sense: GCA GGG GTT AGG CCA ATT AT antisense: CAG GCA AAC TTA CCG AAT CC	203 bp

Figure 3.1 PCR RFLP analysis products of *RAGE* polymorphism -374 A/T, -429 T/C, Gly82Ser and 2184 A/G

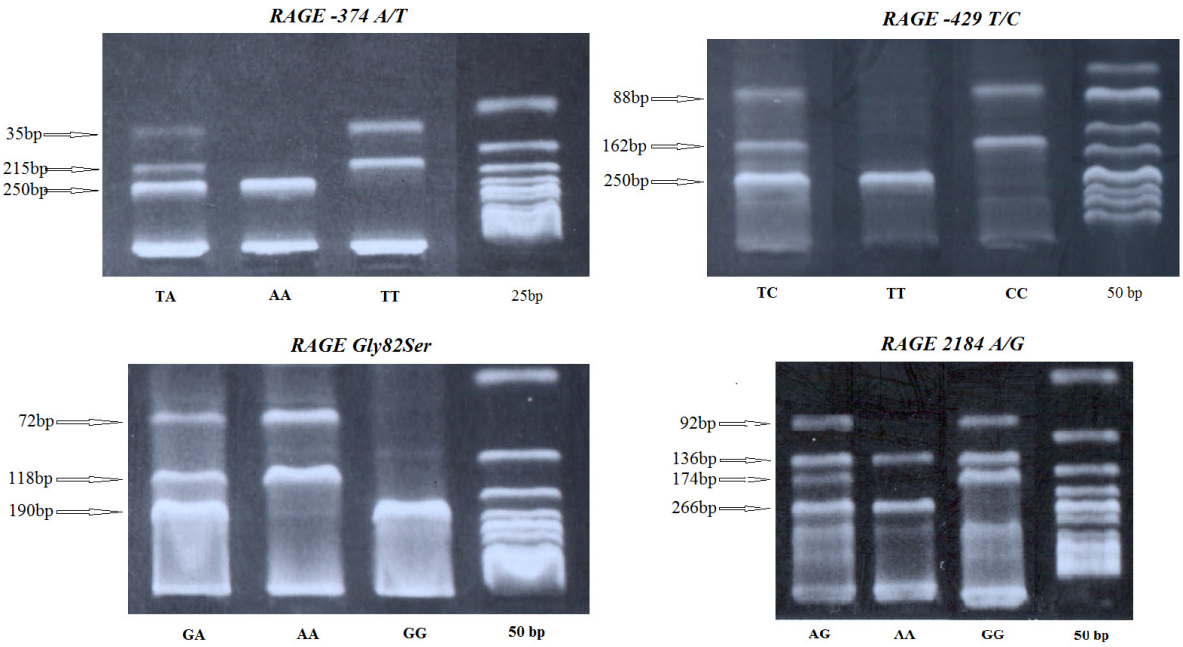
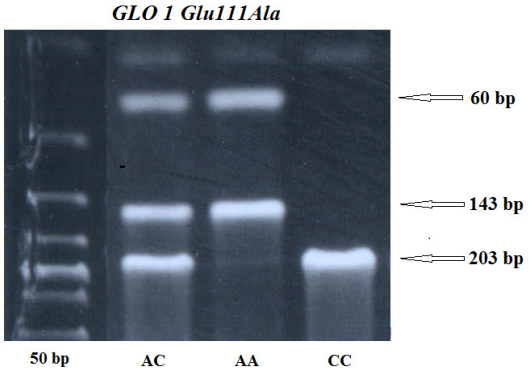


Figure 3.2 PCR RFLP analysis products of *Glyoxalase 1* polymorphism Glu111Ala



Routine Laboratory Parameters

Blood count was assessed by an automated hematological Beckman Coulter LH750 Hematology analyzer (Beckman Coulter, USA). Routine biochemical parameters were measured by commercially available kits using the manufacturer's instructions and certified techniques with automatized modular analyzer (Roche Diagnostics GmbH, Germany)

Statistical Analysis

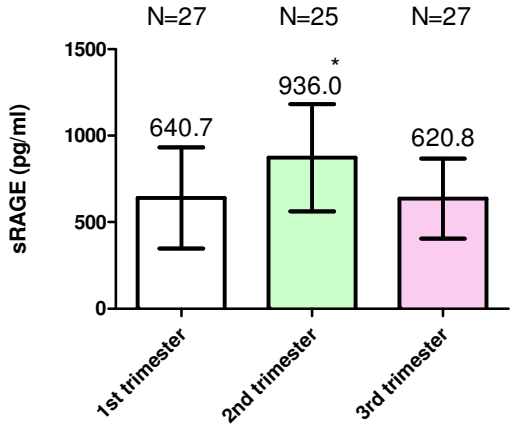
The results of biochemical parameters are expressed as the mean ± the standard deviation. Analysis of group differences was performed by a one-way ANOVA (analysis of variance) test or a Kruskal-Wallis test and an unpaired t-test and Wilcoxon test, where appropriate. Associations between parameters were determined using the Pearson and Spearman correlation coefficients, according to the data distribution. Hardy-Weinberg equilibrium and single polymorphism associations were assessed using the χ^2 test and the Fisher's exact test. Haplotype analysis was used for a more detailed description. Results were considered statistically significant at $p<0.05$.

4. Results

sRAGE analysis

The mean sRAGE serum level during physiological pregnancy is significantly decreased in comparison to the mean sRAGE serum level in non - pregnant controls (727.4±315.4 pg/ml vs. 1937.9±690.8 pg/ml, $p<0.05$). Serum sRAGE levels vary during physiologic pregnancy. They are low during the 1st trimester (640.7±291.4 pg/ml), then in the 2nd trimester, the concentrations rise (936.0±331.1 pg/ml) and fall again in the 3rd trimester and before labor (620.8±225.5 pg/ml). sRAGE serum levels are significantly increased in the 2nd trimester ($p<0.05$) in comparison to the serum levels in the 1st and 3rd trimester as well as in comparison to all groups of healthy pregnant controls.

Figure 4.1 Serum sRAGE levels during physiological pregnancy



Legend: Vertical column bar graph with means and standard deviations, * $p<0.05$, in comparison to 1st trimester, 3rd trimester

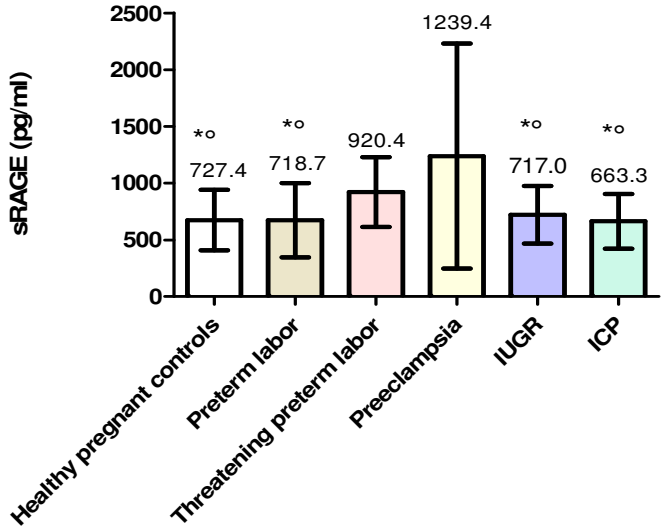
The mean sRAGE serum level in patients with threatening preterm is slightly elevated in comparison to healthy pregnant controls, but the difference is not significant (834.7.6±610.4 pg/ml vs. 727.4±315.4 pg/ml). Patients with preterm labor, who delivered within 24 hours after blood collection, have significantly decreased sRAGE serum concentration in comparison to patients who delivered after 24 hours after blood collection (718.7±395.1 vs. 920.4±307.6 pg/ml, $p<0.05$). sRAGE serum concentrations of patients who delivered soon after enrollment into study are also significantly lower compared to healthy controls in the 2nd trimester (936.0±331.1 pg/ml, $p<0.05$) and do not differ significantly from the sRAGE levels in the controls in the 3rd trimester and before labor (620.8±225.5, $p>0.05$).

The sRAGE serum levels in preterm labor correlate negatively with leukocyte count (r= -0.34, p<0.05) and with absolute neutrophile count (r= -0.34, p<0.05) and do not correlate with other followed parameters.

Serum sRAGE levels in preeclampsia are significantly higher in comparison to healthy pregnant controls as well as to healthy pregnant women in the 1st, 2nd and 3rd trimester (1239.0±991.8 vs. 727.4±315.4 vs. 640.7±291.4 vs. 936.0±331.1 vs. 620.8±225.5 pg/ml, p<0.05).

sRAGE serum levels in patients with preeclampsia correlate positively with proteinuria (grams per day) (r=0.58, p<0.05), with creatinine serum levels (r=0.43, p<0.05) and with uric acid serum levels (r=0.38, p<0.05).

Figure 4.2 Serum sRAGE levels in pathologic pregnancy



Legend: Vertical column bar graph with means and standard deviations, * p<0.05, in comparison to threatening preterm labor, ° p<0.05, in comparison to preeclampsia

sRAGE serum levels in IUGR do not differ significantly in comparison to healthy pregnant controls (717.0±268.7 vs. 727.4±315.4 pg/ml). sRAGE serum levels in IUGR correlate positively with the creatinine serum level (r=0.40, p<0.05) and with uric acid serum levels (r=0.40, p<0.05).

sRAGE serum levels in ICP do not differ significantly from sRAGE serum levels in pregnant controls (663.3±241.2 vs. 727.4±315.4 pg/ml). sRAGE serum levels correlate negatively with ALT serum levels (r= -0.629, p<0.05). AST serum levels tend to correlate with serum sRAGE levels also, but this trend is not significant (r= -0.529, p=0.06).

Genetic analysis

Genotype frequencies of all *RAGE* polymorphisms and *glyoxalase1* polymorphism corresponded to expected frequencies according to Hardy-Weinberg equilibrium (HWE) in all studied groups. Concerning *RAGE* and *GLO1* gene polymorphisms we did not find any differences of allelic or genotype frequencies between studied subgroups.

Table 4.1 Genotype frequencies of studied polymorphisms of *RAGE* and *glyoxalase I* gene in patients with pathological pregnancy and healthy controls, determined frequencies and expected frequencies according to Hardy-Weinberg equilibrium (HWE)

		Patients with pathological pregnancy	HWE	Healthy controls	HWE
***RAGE* -429 T/C**	**TT**	68.5	67.4	65.1	66.2
(rs1800625)	**TC**	27.3	29.4	32.5	30.3
(%)	**CC**	4.2	3.2	2.4	3.5
***RAGE* -374 A/T**	**TT**	39.4	40.5	39.7	42.3
(rs1800624)	**AT**	48.5	46.3	50.8	45.5
(%)	**AA**	12.1	13.2	9.5	12.2
RAGE Gly82Ser	**GG**	96.4	95.8	93.7	93.8
(rs2070600)	**GA**	3.0	4.1	6.3	6.1
(%)	**AA**	0.6	0.1	0	0.1
***RAGE* 2184 A/G**	**AA**	67.3	67.9	65.9	66.2
(rs13209119)	**AG**	30.3	29.0	30.9	30.3
(%)	**GG**	2.4	3.1	3.2	3.5
Glo1 Glu111Ala	**AA**	30.9	28.2	34.9	32.2
(rs4746)	**AC**	40.6	46.0	43.7	49.1
(%)	**CC**	28.5	25.8	21.4	18.7

Legend: the differences are not significant

We did not find any significant difference in haplotype frequencies between studied groups as well.

Concerning relationships of studied polymorphisms to sRAGE serum levels, we discovered that healthy pregnant women with GA genotype of *RAGE* Gly82Ser polymorphism had significantly lower serum levels of sRAGE in comparison to healthy controls with GG genotype (483.0±104.0 vs. 692.0±262.0 pg/ml, $p<0.05$) The similar trend

was discovered in patients with pathological pregnancy, however the difference was not significant (565.2±118.0 vs. 908.1±698.2 pg/ml, p=0.08). We did not find any other association of *RAGE* polymorphisms or *Glyoxalase 1* polymorphism with sRAGE serum levels.

Table 4.2 Genotype and allelic frequencies in studied subgroups

			Pregnant controls N=120	Threatening preterm labor N=99	Preeclampsia N=35	IUGR N=22	ICP N=14
RAGE -429 T/C (rs1800625)	**Alleles (%)**	**T**	82.1	72.9	81.0	80.5	76.5
		C	17.9	27.1	19.0	19.5	23.5
	Genotypes (%)	**TT**	65.1	64.6	77.1	76.5	71.4
		TC	32.5	30.3	20.0	20.6	21.4
		CC	2.4	5.1	2.9	2.9	7.2
RAGE -374 A/T (rs1800624)	**Alleles (%)**	**T**	65.1	59.5	55.8	57.7	65.0
		A	34.9	40.5	44.2	42.3	35.0
	Genotypes (%)	**TT**	39.7	39.3	34.3	35.3	50.0
		TA	50.8	49.5	48.6	52.9	42.9
		AA	9.5	11.1	17.1	11.8	7.1
RAGE Gly82Ser (rs2070600)	**Alleles (%)**	**G**	96.7	96.1	100	97.1	93.3
		A	3.3	3.9	0.0	2.9	6.7
	Genotypes (%)	**GG**	93.7	96.0	100	97.0	92.9
		GA	6.3	3.0	0.0	3.0	7.1
		AA	0	1.0	0.0	0.0	0
RAGE 2184 A/G (rs13209119)	**Alleles (%)**	**A**	81.3	73.6	80.0	75.6	77.8
		G	18.7	26.4	20.0	24.4	22.2
	Genotypes (%)	**AA**	65.9	65.7	74.2	67.6	71.4
		AG	30.9	30.3	25.8	32.4	28.6
		GG	3.2	4.0	0.0	0.0	0.0
Glo1 Glu111Ala (rs4746)	**Alleles (%)**	**A**	56.7	48.6	57.4	52.9	57.9
		C	43.3	51.4	42.6	47.1	42.1
	Genotypes (%)	**AA**	34.9	27.3	42.9	29.4	42.9
		AC	43.7	41.4	34.3	50.0	35.7
		CC	21.4	31.3	22.8	20.6	21.4

Legend: the differences are not significant

5. Discussion

In this study, I have demonstrated changes of serum sRAGE levels during physiological pregnancy - lower levels in the 1st trimester, elevated levels in the 2nd trimester and decreased levels in the 3rd trimester and before labor. I have shown altered sRAGE serum levels in patients with symptoms of threatening preterm levels, especially the difference between sRAGE serum concentration between patients, who delivered prematurely immediately after enrollment into the study and patients, whose labor was postponed. I have confirmed increased sRAGE serum levels in patients with preeclampsia. I have not shown significant alteration in sRAGE serum levels in patients with intrauterine growth retardation and intrahepatic cholestasis in pregnancy.

Concerning *RAGE* polymorphisms (-429T/C, -374T/A, Gly82Ser (557G/A), 2184A/G) and *glyoxalase 1* polymorphism (Glu111Ala - 419A/C) I have not discovered any significant differences in allelic, genotype or haplotype frequencies among studied groups. However, the association of genotype 557GA of *RAGE* Gly82Ser (557G/A) polymorphism with a decreased serum sRAGE level was confirmed in accordance with previous studies.

sRAGE Analysis

Pregnancy is a state when the women's body undergoes delicate changes in the metabolism and in immunological, hormonal and other systems. Many studies have shown proof of increased oxidative status in physiologic and pathologic pregnancy (Fialová L. et al., 2006), as well as changes in the protective mechanisms (Kharb S., 2000). This study shows low sRAGE serum levels at the beginning of pregnancy in comparison to healthy non-pregnant women, which is likely a result of sRAGE consumption as a reaction to the increasing oxidative stress. The elevation of sRAGE concentration in the 2nd trimester might be explained by compensatory processes to accelerated oxidative stress (Toescu V. et al., 2002). In the 3rd trimester proinflammatory and inflammatory processes result in labor (Norman JE. et al., 2007). sRAGE production in the 3rd trimester is probably not sufficient and serum sRAGE levels are diminished by being used up. Nevertheless we have to count delicate changes in the mother's organism during pregnancy, especially changes in the renal system have to be considered as it is proven that sRAGE is present in the renal tubus and renal tubular lumen (Cheng C. et al., 2005).

The study (Germanová A. et al., 2010) presented for the first time the dynamics of sRAGE throughout physiological pregnancy. A latter study of Kwon (Kwon JT et al., 2011),

focused on levels of esRAGE during the normal pregnancy and showed continuously decreasing esRAGE maternal serum levels and decreasing esRAGE/sRAGE ratio throughout the pregnancy. The difference might be explained by a different population and the influence of a diverse RAGE polymorphism distribution in the population.

Inflammatory processes, which are considered as the main etiology factor of preterm labor, are clearly associated with sRAGE (Meijer B. et al., 2014). sRAGE and its association with preterm labor has been already studied. Buhimschi et al. (Buhimschi IA. et al., 2007) showed that sRAGE amniotic fluid are not influenced by intraamniotic infection. On the other hand, Romero et al. (Romero R. et al., 2008) detected that sRAGE amniotic fluid levels are increased in patients with intraamniotic infection. My study focused on sRAGE serum levels in patients with preterm labor. Our first results (Hájek Z. et al., 2008) showed that sRAGE serum levels are increased in patients with threatening preterm labor in comparison to healthy pregnant controls. However, the following study (Germanová A. et al., 2010) presented decreased sRAGE serum levels in patients with preterm labor compared to healthy pregnant women. The difference is caused by the timing of sRAGE examination. The first study focused mainly on patients with the first symptoms of preterm labor, while the following one was aimed at patients with preterm labor in progress. The sRAGE serum level decreases towards preterm delivery, it is lower in patients24 hours prior to preterm labor than in patients with symptoms of threatening preterm labor who did not deliver within 24 hours. The serum concentration of sRAGE immediately before preterm labor seems to be very similar to the serum concentration of sRAGE before term delivery. Preterm labor trigger factors activate similar processes that initiate term labor. Parts of both are inflammatory actions and oxidative stress (Lee SE. et al., 2008, Romero R. et al., 2006) and thus, sRAGE can be depleted towards to preterm as well as term labor. Similar results to ours showed a latter study by Bastek (Bastek JA. et al., 2012), which in accordance to our study described a significantly low serum sRAGE concentration in patients in preterm labor.

The focus on the receptor for advanced glycation end products in preeclampsia is clearly understandable. The pathogenesis of preeclampsia is still not well understood. Interestingly, sRAGE levels are decreased in patients with arterial hypertension (Geroldi D. et al., 2005) however, their concentrations are very high in patients with proteinuria (Tan KC. et al., 2006) or chronic renal diseases (Kalousová M. et al., 2006). Several studies including ours described elevated maternal sRAGE levels in women with preeclampsia (Fasshauer M. et al., 2008, Germanová A. et al., 2010). As preeclampsia is a state defined by hypertension, we also would expect decreased sRAGE levels in preeclampsia. Nevertheless sRAGE maternal serum

levels are significantly high in preeclampsia, so the role of the impaired renal function is likely to be relevant here. However, it is not clear whether this elevation is caused by the decreased elimination of sRAGE by the kidneys or up-regulation of sRAGE production. An increased esRAGE/sRAGE ratio in preeclampsia (Kwon JT. et al., 2011), indicates the importance of sRAGE up-regulation.

Maternal serum sRAGE levels in patients with intrahepatic cholestasis have only been described by our study (Germanová A. et al., 2012). sRAGE serum levels do not differ in comparison to healthy pregnant controls. However, sRAGE negatively correlates with serum ALT levels. The secondary bile acid, deoxycholic acid, which is also elevated in patients with ICP, promotes the production of one of the RAGE ligands, HMGB1 protein (Fujii K. et al., 2009). Assuming that increased ALT and AST serum levels reflect increased levels of bile acids, negative correlation of sRAGE with ALT is caused by consumption of sRAGE.

Maternal serum sRAGE levels in women with IUGR do not differ from healthy pregnant women. Causes of intrauterine growth restriction are different and comprise fetal genetic abnormalities, placental dysfunction and maternal diseases. It is believed that a common sign of IUGR is oxidative stress (Karowicz-Bilinska A. et al., 2007). Choi et al. showed that hypoxically regulated protein N-myc-downstream regulated gene 1 (NDRG1) is expressed more in placentas of patients with preeclampsia than in placentas of IUGR (Choi SJ. et al., 2007). We could speculate that oxidative stress in IUGR is not so massive as in preeclampsia, so sRAGE levels are not affected.

RAGE and Glyoxalase 1 Polymorphisms Analysis

Considering all previous facts about the importance of RAGE and sRAGE in the pathogenesis of pregnancy associated diseases, the question about the significance of *RAGE* genetics and *RAGE* polymorphisms in these diseases arises. My work has focused on four *RAGE* polymorphisms and their association mainly with preterm labor. Groups of patients with preeclampsia, ICP and IUGR were not big enough to make a significant statistical conclusion. The glimpse of the connection of *RAGE* polymorphisms to these diseases was also done.

RAGE polymorphisms and their association with the susceptibility to chronic diseases have been studied intensively.

Functional *RAGE* -374 T/A and -429 T/C polymorphisms in the gene promoter affect the transcriptional activity of the *RAGE* gene (Hudson BI. et al., 2001).

-429T/C *RAGE* polymorphism is associated with acute inflammatory diseases (Zeng L. et al., 2012) and also with autoimmune diseases such systemic lupus erytematodes and lupus nephritis (Martens HA. et al., 2012). *429T/C* polymorphism is linked to diabetes mellitus (Picheth G. et al., 2007) and its complications (Hudson BI. et al., 2001).

More studies until now have focused on the other *RAGE* gene promoter polymorphism -374 T/A. Several studies have affirmed the association of this polymorphism with chronic inflammatory diseases such as systemic lupus erytematodes (Martens HA. et al., 2012), Crohn's disease (Dabritz J. et al., 2011) and multiple sclerosis. *RAGE* -374 T/A polymorphism is also linked to diabetes mellitus and its complications (Kawai T. et al., 2013). Also, the study by Falcone C. et al. showed that non-diabetic patients with genotype AA of *RAGE* -374 T/A polymorphism had significantly less affected coronary arteries with severe atherosclerosis (Falcone C. et al., 2005).

RAGE Gly82Ser (557G/A) polymorphism in exon 3 is connected with amino-acid change at position 82 of the protein. Minor allele A evokes an exchange of glycine for serine. This exchange increases RAGE's ability to bind AGEs (Osawa M. et al., 2007). Several studies (Germanová A. et al., 2012, Gaens KH. et al., 2009), including ours, confirmed that decreased sRAGE levels are associated with the minor allele A also. The mechanism of this finding is currently not clear. We could speculate that the increased ability of RAGE to bind AGEs caused by the A allele, blocks the cleavage of cRAGE, component of sRAGE. Genotype AA of the polymorphism is associated with elevated CRP and TNF serum levels in non-obese, non-diabetic Korean subjects (Jang Y. et al., 2007). Also the A allele is more frequent in patients with rheumatoid arthritis (Hofmann MA. et al., 2002). Concerning cardiovascular diseases, the A allele is associated with ischemic stroke (Cui X. et al., 2013).

RAGE 2184 A/G polymorphism is located in intron 8. It is located near the splicing site, so it most likely influences alternative splicing of RAGE. Thus, it might affect sRAGE levels. The study by Kalousová et al. (Kalousová M. et al., 2007) supports this assumption. The study shows an elevated sRAGE serum concentration in hemodialyzed patients with genotype GG, however, we have to consider an altered renal function in the study also. The polymorphism is associated with systemic lupus erytematodes (Martens HA. et al., 2012). The GG genotype is also associated with an elevated oxidative status (Kaňková K. et al., 2001).

Event though there are many studies which approve the significance of *RAGE* polymorphisms in diabetes mellitus, inflammatory, cardiovascular and renal disease, the only study by Santos (Santos IC. et al., 2010) focused on gestational disease, precisely on

gestational diabetes, but the study did not show any association between the studied promotor polymorphisms, *RAGE* -429 T/C and *RAGE* -374T/A, and gestational diabetes.

My work (Germanová A. et al., 2012) studied four above mentioned polymorphisms in pregnancy associated diseases. Despite the evidence that these *RAGE* polymorphisms are linked with acute or chronic inflammatory diseases, my work did not prove any association between any of the studied *RAGE* polymorphisms and preterm labor, whose main cause is infection and inflammation.

The connection of *RAGE* polymorphisms to hypertension and proteinuria is also known, but in our small group of patients with preeclampsia, we did not describe any linkage to *RAGE* polymorphisms. Another study with a larger number of subjects would be interesting to elucidate meaning of *RAGE* polymorphisms in pathogenesis of preeclampsia, because role of RAGE in preeclampsia is undeniable.

Our study also looked at *RAGE* polymorphisms in IUGR and ICP and did not find any association. However, the studied groups were too small to make any significant conclusion.

The role of glyoxalase 1 in pathological pregnancy has not been discussed much. Only the study by Sankaralingam (Sankaralingam S. et al, 2009) focused on glyoxalase 1 in pathological pregnancy and showed that glyoxalase 1 is reduced in preeclampsia.

My work has focused on the connection between *GLO1* Glu111Ala polymorphism and pregnancy complication. The polymorphism located in the 4^{th} exon causes amino acid change in the polypeptide chain (Glu111Ala). This change causes decreased activity of glyoxalase 1 (Barua M. et al., 2011) and an accumulation of AGEs precursors and their pathological effect.

Our study did not find any connection between *GLO1* Glu111Ala polymorphism and preterm labor. There is no other study focusing on the role of AGEs or glyoxalase 1 in preterm labor. However, several studies have proved the role of RAGE and sRAGE in preterm delivery. Despite the evidence about the connection between the RAGE axis and the glyoxalase system, my work has not found the importance of *glyoxalase 1* in preterm labor.

GLO1 419 A/C polymorphism might be associated with preeclampsia, while it was described that preeclampia is associated with elevated AGEs concentrations and decreased expression of glyoxalase 1 enzyme (Sankralingam S. et al, 2009). However, our study did not find an association between *GLO1* 419A/C polymorphism and preeclampsia, but our study group was too small for a significant statistic conclusion.

Our study did not find any connection between *GLO1* Glu111Ala polymorphism and IUGR or ICP, but both studied groups were too small, so another study to clarify these results is needed.

6. Conclusion

Receptor for advanced glycation end products, its soluble form and glyoxalase 1 take part in the pathogenesis of chronic diseases associated with altered oxidative status and microinflammation. Oxidative stress and microinflammatory processes are as well characteristic for physiologic pregnancy and even more for pregnancy complications.

Serum sRAGE in physiologic pregnancy and pregnancy induced diseases

My research showed that serum sRAGE levels in healthy pregnant controls are significantly decreased in comparison to healthy non-pregnant subjects. However serum sRAGE levels dynamically change during physiological pregnancy. Serum sRAGE levels steeply decrease in the 1st trimester of physiologic pregnancy and continue with elevation in the 2nd trimester and a slow decrease in the 3rd trimester and before labor.

Concerning pathological pregnancy serum sRAGE levels are decreased during the preterm labor in comparison to threatening preterm labor and in comparison to healthy pregnant controls. sRAGE correlates with leukocyte count in preterm labor. Serum sRAGE concentrations are elevated in patients with preeclampsia. sRAGE positively correlates with proteinuria, serum uric acid and serum creatinine. Serum sRAGE concentrations in IUGR and ICP are not affected, but sRAGE correlates negatively with serum ALT levels in ICP and it correlates positively with serum uric acid and serum creatinine in IUGR.

Significance of RAGE and GLO1 polymorphisms in pregnant women

Four *RAGE* (*RAGE* -429T/C, *RAGE* -374 T/A, *RAGE* Gly82Ser (557G/A), *RAGE* 2184A/G) polymorphisms and one *Glyoxalase 1* polymorphism (*GLO1* Glu111Ala (419A/C)) were analyzed in this study. Despite the significant changes in sRAGE levels in pregnancy complications and association of studied polymorphisms with the susceptibility to chronic diseases, my work did not find any genotype or allelic distribution differences between women with pregnancy induced pathologies and women with physiological pregnancy. Similarly my work did not discover any connection of *GLO1* Glu111Ala (419A/C) polymorphism with pregnancy complications.

The results of my work could bring better insight to the pregnancy and its complications. Better understanding of physiologic pregnancy and pregnancy induced diseases could improve screening of pregnancy associated diseases and prenatal care.

7. References

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List of original articles

1. Publications related to the thesis

a) with IF

1. ***Germanová A.***, Muravská A., Jáchymová M., Hájek Z., Koucký M., Mestek O., Zima T., Kalousová M.: Receptor for advanced glycation end products (RAGE) and glyoxalase I gene polymorphisms in pathological pregnancy. Clin Bioch., 2012; 45(16-17): 1409-1414.
IF = 2.076

2. ***Germanová A.***, Koucký M., Hájek Z., Pařízek A., Zima T., Kalousová M.: Soluble receptor for advanced glycation end producats in physiological and pathological pregnancy. Clin. Biochem., 2010; 43(4-5): 442-446. **IF=2.019**

3. Hájek Z., ***Germanová A.***, Koucký M., Zima T., Kopecký P., Vítková M., Pařízek A., Kalousová M.: Detection of feto-maternal infection/inflammation by the soluble receptor for advanced glycation end products (sRAGE): results of pilot study. J. Perinat. Med., 2008; 36(5): 399-404. **IF=1.101**

2. Other publications:

a) with IF

1. Koucký M., Malíčková K., Cindrová-Davies T., ***Germanová A***., Pařízek A., Kalousová M., Hájek Z., Zima T.: Low levels of circulating T-regulatory lymphocytes and short cervical length are associated with preterm labor. J Reprod Immunol., 2014; Epub ahead of print. **IF=2.342**

2. Muravská A., ***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(17-18): 1380 - 1384. **IF=2.043**

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5. Hill M., Pařízek A., Jirásek JE., Jirovská M., Velíková M., Dusková M., Klímková M., Pašková A., Žižka Z., ***Germanová A.***, Kalousová M., Stárka L.: Is maternal progesterone actually independent of the fetal steroids? Physiol. Res., 2010; 59 (2): 211 - 224. **IF=1.430**

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b) without IF

1. Koucký M., **Germanová A**., Hájek Z., Pařízek A., Zima T., Kalousová M., Kopecký P.: Prenatal and perinatal management of preterm labour. Prague Med Rep. , 2009; 110(4), 269 - 277.

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