ОРИГИНАЛЬНЫЕ СТАТЬИ

N.V. ANTIPOVA[1], S.A. LEVAKOV[2], N.A. SHESHUKOVA[2],
E.A. OBUKHOVA[2], M.S. PAVLYUKOV[1], M.I. SHAKHPARONOV[1]

PLACENTAL EXPRESSION LEVEL OF THE *PON1, PON2,* AND *PON3* GENES IN PATIENTS WITH UNCOMPLICATED PREGNANCY AND PREECLAMPSIA

[1]Academicians M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia
[2]I.M. Sechenov First Moscow State Medical University (Sechenov University), Ministry of Health of Russia

Objective. *To determine the expression level of the genes encoding the paraoxonase family enzymes (PON1, PON2, and PON3) in the placentas of women with uncomplicated pregnancy and in the development of preeclampsia.*
Materials and methods. *The investigation enrolled 26 pregnant women aged 24 to 35 years, including 14 women with normal pregnancy and 12 with preeclampsia. The expression of the PON1, PON2, and PON3 genes was analyzed by real-time PCR using the primers specific to these genes.*
Results. *Women whose pregnancy was complicated by preeclampsia showed a significant decrease in the placental expression of the PON2 gene. The lowest PON2 gene expression was found in the placentas of women with severe preeclampsia. There were no significant differences in the placental expression level of the PON1 and PON3 genes in women with preeclampsia compared to healthy women.*
Conclusion. *In women with preeclampsia, the placental PON2 gene expression decreases; however, the expression level of the PON1 and PON3 genes does not differ from that of the PON1 and PON3 genes in the placentas of patients with physiological pregnancy. The placental expression of the PON2 gene depends on the severity of preeclampsia; it is lower in severe preeclampsia than in moderate preeclampsia.*

Keywords: *paraoxonase, oxidative stress, moderate preeclampsia, severe preeclampsia.*
The investigation was performed under Russian Science Foundation Grant 16-14-10335.

Authors declare lack of the possible conflicts of interests.

For citations: Antipova N.V., Levakov S.A., Sheshukova N.A., Obukhova E.A., Pavlyukov M.S., Shakhparonov M.I. Placental expression level of the PON1, PON2, and PON3 genes in patients with uncomplicated pregnancy and preeclampsia. Akusherstvo i Ginekologiya/Obstetrics and Gynecology. 2018; (7): 40-4. (in Russian)
https://dx.doi.org/10.18565/aig.2018.7.40-44

The oxidative stress plays a central role in pathogenesis of many obstetric complications [1]. Evolutionarily formed redox-homeostatic system includes reactive oxygen species and antioxidants. Reactive oxygen species (ROS) are constantly generated in cells and play a key role in maintaining the chemical environment of each cell in the body [1,2]. Antioxidant system limits the formation of free radicals under physiological conditions. A balance between free radicals and antioxidants is necessary for proper physiological function. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues. Oxidative stress is involved in many typical pathological processes leading to the development of diseases, as well as obstetric complications [1]. Cytotrophoblastic invasion is the process of migration of trophoblast cells into the endometrium and myometrium that determines the course of pregnancy. When this mechanism is compromised, incomplete remodeling of spiral arteries occurs with the further development of hypoxia, placental insufficiency and preeclampsia [3]. Preeclampsia is currently defined as a multisystem pathological condition that occurs in the second half of pregnancy (after 20 weeks' gestation), characterized by hypertension in combination with proteinuria (0.3g/l in daily urine), often with edema and manifestations of the multiple-organ dysfunction syndrome. Despite the greatest achievements in modern medicine, this complication of pregnancy is still one of the leading causes of maternal and perinatal mortality [4-6]. In *early stages* the *failure of trophoblast invasion* and spiral artery transformation leads to lumen narrowing and the development of placental ischemia. At the subcellular level, hypoxia activating the expression of xanthine oxidase and NADPH oxidases, which are the main sources of the superoxide anion (O_2^-), triggers the mechanism of oxidative stress development. The second stage is characterized by functional disturbances of the endothelium and multisystem inflammatory response. At present, considerable attention is being *paid to the study* of the oxidative stress induction mechanisms and the search for early prognostic criteria for the development of preeclampsia. The level of antioxidant defense genes expression (glutathione peroxidase —GPX1 and glutathione reductase - GSR) in the placenta has been studied during preeclampsia [7].

Enzymes of the paraoxonase family (*PON1, PON2* and *PON3*) have ability to neutralize free oxygen radicals,

and protect lipids of cell membranes from peroxidation. Moreover, they are known to have powerful anti-inflammatory effects, and regulate apoptosis processes. *PON1* and *PON3* enzymes are synthesized primarily in hepatocytes, and are secreted to the blood plasma in a complex with high-density lipoproteins. The *PON2* enzyme is located exclusively intracellularly and is not detected in the plasma. Paraoxonases belong to the family of *hydrolases,* according to their chemical properties [8]. They are able to hydrolyze oxidized phospholipids of cell membranes, estrogen esters, organophosphorus compounds, unsaturated aliphatic esters, aromatic esters of carboxylic acids and acylgoserine lactones, which are signal molecules of quorum sencing of gram-negative bacteria. Thus, they may play a potential protective role in the development of obstetric complications. At present, the level of expression of paraoxonases in placentas under various pathological conditions is insufficiently studied.

Objective**.** To examine mRNA expression of *PON1, PON2* and *PON3* in placentas obtained from women with normal pregnancy and with pre-eclampsia.

Materials and methods

A retrospective study was conducted with the participation of 26 pregnant women at 34-40 weeks' gestation: group I (main) included the patients whose pregnancy was complicated by preeclampsia (n = 12), group II (comparison) comprised the patients with normal pregnancy (n = 14). The average age of pregnant women was 28 ± 4 years. All patients enrolled in the *study* were comparable in terms of parity, somatic and gynecological status.

Criteria for inclusion in the study were reproductive age, delivery by cesarean section, absence of severe neuroendocrine somatic pathology, single natural pregnancy.Exclusion criteria were acute inflammatory processes, presence of oncological diseases in the anamnesis.

In group I eight patients had pregnancy with moderate preeclampsia, four patients had severe preeclampsia. The diagnosis of moderate preeclampsia was established due to the following criteria: arterial hypertension (systolic blood pressure 140-159mm Hg or diastolic blood pressure 90-109mm Hg in women with gestation age more than 20 weeks and normal blood pressure in the medical history); proteinuria ⩾ 0.3g/l in a daily urine sample. The diagnosis of severe preeclampsia was *made on* the basis of the following clinical and laboratory indicators: the diastolic blood pressure ⩾110mm Hg, systolic blood pressure ⩾160mm Hg and level of daily proteinuria > 5g/l and symptoms of multiple organ failure. Patients with preeclampsia complicated by acute fetal hypoxia were delivered by emergency cesarean section. In group II *indications* for planned cesarean *delivery* included a large fetus in combination with a breech presentation. In all cases cesarean section was performed typically and the postoperative period proceeded without any complications. The average APGAR score was 7/8 for group I, and 8/9 for group II. All patients included in the study were discharged in a satisfactory condition on the 4th day after the operation.

Isolation and analysis of mRNA

Placentas were obtained directly after delivery, washed in water and the amnion was removed. The central parts of the placentas (1x1 cm) were obtained. When sampled, 1 ml of Trisol solution was added to the samples, and the samples were homogenized and subjected to a quick freeze at -80 ° C. Total mRNA was isolated according to the Matz protocol, 2002, the first cDNA chain was synthesized according to the Evrogen protocol for subsequent amplification. The expression levels of PON1, PON2 and PON3 were determined by real-time polymerase chain reaction using a LightCycler 96 Real-Time PCR System (Roche) with primers specific for PON1, PON2 and PON3 (Table).

Table. The nucleotide sequences of specific primers used in the study

Gene	Forward primer	Reverse primer
PON1	CCGAGAGGTACAACCCGTAG	TGT GAA TGT GCT AAT CCC ATG
PON2	CATCTGGGTAGGCTGTCATC	TTGGAGAACAGACCCATTGT
PON3	TCCCCACATGAAGTCCACTG	TGG TCT GCT GAG ACT GTG AT

Expression data were normalized to Actin β expression levels.

Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics 20. The arithmetic mean and the standard *error* of the *mean* were determined for all groups. Comparison of two groups was performed by the Mann Whitney test. Differences were considered statistically significant at a threshold confidence level of $p<0.05$. All results are presented as M ± SEM, where M is the mean value, SEM is the standard error of the mean.

Results and discussion

PON1 expression was undetectable in the samples of the placenta from both groups. This result is *presumably due to the fact* that the expression of the PON1 gene and the synthesis of the PON1 enzyme mainly occur in hepatocytes. On the other hand, there was a significant decrease in PON2 expression in placentas of the women, whose pregnancy was complicated by preeclampsia (Fig. 1) when compared to the control group ($p<0.05$, nonparametric

Figure 1. PON-2 relative gene expression in placenta (physiological pregnancy 40,53±17,45, preeclampsia 4,82±3,29)

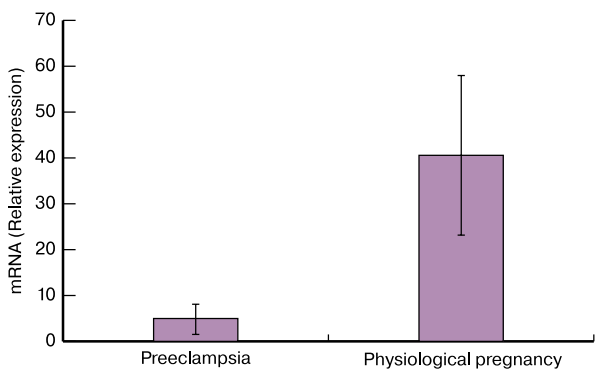
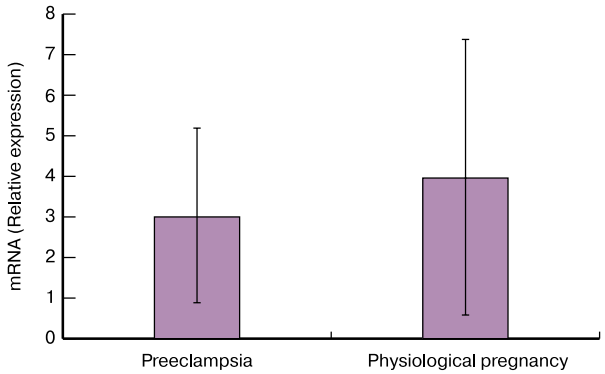


Figure 2. PON-3 relative gene expression in placenta (physiological pregnancy 3,97±3,46, preeclampsia 2,99±2,18)



Mann-Whitney test). The placenta samples obtained from women with severe and moderate preeclampsia were pooled in one group due to the rare occurrence of severe preeclampsia and a small number of placenta samples with this pathology. However, the lowest level of PON2 gene expression was detected in the placenta of patients with severe preeclampsia (norm 40.53 ± 17.45, preeclampsia 4.82 ± 3.29, severe preeclampsia 1.02 ± 0.25). Thus, the expression level of the PON2 gene may serve as an indicator of the presence of oxidative stress and reflect the degree of severity of preeclampsia, therefore, it can be a candidate for the role of a molecular genetic marker in this pathological process. Our hypothesis was confirmed by the results of other studies that demonstrated a significant decrease in the amount and activity of PON2 and PON3 enzymes in the placenta of women with preeclampsia in comparison with healthy patients [9].

When we compared the expression level of the PON3 gene in the same samples, no statistically significant differences were detected ($p > 0.05$, the nonparametric Mann-Whitney test). However, there was a certain tendency for downregulation of the PON3 gene in the placenta of patients whose pregnancy was complicated by preeclampsia (Fig. 2).

Conclusion

Our research has shown a significant decrease in the level of placental expression of the PON2 gene in patients with preeclampsia, while in women with severe preeclampsia this indicator was even lower than in women with moderate preeclampsia. *These results suggest* that a low level of expression of the PON2 gene is a possible prognostic marker of the development of preeclampsia and can be used to predict pregnancy outcomes. In the course of the study, it was shown that the level of expression of PON3 does not significantly differ in patients with normal pregnancy and those with pre-eclampsia. In both groups, placental expression of the PON1 gene was not detected, but according to the previous studies, the serum activity of this enzyme was decreased in pregnant women with preeclampsia compared with healthy patients [10]. Therefore, further research in this area is required to improve the early diagnosis of preeclampsia, as well as predict the course and outcome of pregnancy.

References

1. *Duhig K., Chappell L.C., Shennan A.H.* Oxidative stress in pregnancy and reproduction. Obstet. Med. 2016; 9(3):113-6.
2. *Droge W.* Free radicals in the physiological control of cell function. Physiol. Rev. 2002; 82(1): 47-95.
3. *Lim K.H., Zhou Y., Janatpour M., McMaster M., Bass K., Chun S.H., Fisher S.J.* Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia. Am. J. Pathol. 1997; 151(6): 1809-18.
4. Hypertensive disorders during pregnancy, during childbirth and the postpartum period. Preeclampsia. Eclampsia. Clinical recommendations (protocol of treatment). Approved. The Ministry of Health of the Russian Federation of June 7, 2016 No. 15-4 / 10 / 2-3483. Moscow; 2016. 40p. (in Russian)
5. *Eiland E., Nzerue C., Faulkner M.* Preeclampsia 2012. J Pregnancy. 2012; 2012: 586578.
6. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. American College of Obstetricians and ynecologists. Hypertension in pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet. Gynecol. 2013; 122(5): 1122-31.
7. *Sukhikh G.T., Krasnyi A.M., Kan N.E., Maiorova T.D., Tyutyunnik V.L., Khovkhaeva P.A., Sergunina O.A., Tyutyunnik N.V., Gracheva M.I., Vavina O.V., Ozernyuk N.D., Boris D.A.* Placental apoptosis and antioxidant defense enzyme gene expression in preeclampsia. Akusherstvo i Ginekologiya/Obstetrics and Gynecology. 2015; 3: 11-5. (in Russian)
8. *Efimtseva E.A., Chelpanov T.I.* Paraoxonase: molecular-genetic aspects and clinical significance Uspehi sovremennoy biologii. 2012; 132 (3): 282-96. (in Russian)
9. *Alwarfaly S.* The role of the paraoxonases in pre-eclampsia and labour. MRes thesis, University of Glasgow. February, 2015. Available at: http://theses.gla.ac.uk/6167/
10. *Bargale A.B., Ganu J.V., Trivedi D.J., Kamble P.S., Mudaraddi R.* Serum superoxide dismutase and paraoxonase-1 activity in preeclampsia patients. Int. J. Pharm. Biosci. 2011; 2(4): 705-9.

Received 30.11.2017
Accepted 22.12.2017

About the authors:
Nadezhda V. Antipova, PhD, research fellow, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences; associate professor,Department of Pharmaceutical and Toxicological Chemistry of the Peoples' Friendship University of Russia. 117997, Russia, Moscow, Miklukho-Maklaya str. 16/10. Tel: +74953350100. E-mail: office@ibch.ru
Sergey A. Levakov, MD, PhD, professor, head of the Department of Obstetrics and Gynecology, I.M. Sechenov First Moscow State Medical University (Sechenov University). 119991, Russia, Moscow, Trubetskaya str. 8/2. Tel.: +74956091400 (ext.2234).
Natalya A. Sheshukova, MD, PhD, professor, Department of Obstetrics and Gynecology, I.M. Sechenov First Moscow State Medical University (Sechenov University). 119991, Russia, Moscow, Trubetskaya str. 8/2. Tel.: +74956091400 (ext.2234).
Elizaveta A. Obukhova, graduate student, Department of Obstetrics and Gynecology, I.M. Sechenov First Moscow State Medical University (Sechenov University). 119991, Russia, Moscow, Trubetskaya str. 8/2. Tel.: +74956091400 (ext.2234). E-mail: liza_obukhova@mail.ru
Marat S. Pavlyukov, PhD, research fellow, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences. 117997, Russia, Moscow, Miklukho-Maklaya str. 16/10
Mikhail I. Shakhparonov, Doctor of Chemical Sciences, leading researcher, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences. 117997, Russia, Moscow, Miklukho-Maklaya str. 16/10