UDC – 618.3-06:616.8-009.24.
DOI – http://dx.doi.org/10.14300/mnnc.2015.10033
ISSN – 2073-8137

IMMUNOHISTOCHEMICAL EXPRESSION OF MATRIX METALLOPROTEINASE-9 AND TISSUE INHIBITOR OF METALLOPROTEINASE-1 IN PLACENTAL TISSUE IN LATE SEVERE PREECLAMPSIA

Milovanov A. P.[1], Dubovoy A. A.[2], Aksenenko V. A.[2], Rasstrigina I. M.[1], Fokina T. V.[1], Nikolskaya T. N.[2]

[1] Research Institute of Human Morphology, Moscow, Russian Federation
[2] Stavropol State Medical University, Stavropol, Russian Federation

ОЦЕНКА ИММУНОГИСТОХИМИЧЕСКОЙ ЭКСПРЕССИИ МАТРИКСНОЙ МЕТАЛЛОПРОТЕИНАЗЫ 9 ТИПА И ЕЁ ИНГИБИТОРА 1 ТИПА В ПЛАЦЕНТАРНОЙ ТКАНИ ПРИ ПОЗДНЕЙ ТЯЖЕЛОЙ ПРЕЭКЛАМПСИИ

А. П. Милованов[1], А. А. Дубовой[2], В. А. Аксененко[2], И. М. Расстригина[1], Т. В. Фокина[1], Т. Н. Никольская[2]

[1] НИИ морфологии человека РАН, Москва, Российская Федерация
[2] Ставропольский государственный медицинский университет, Ставрополь, Российская Федерация

It was carried out histochemical study of placentas of healthy pregnant women and pregnant women with late severe preeclampsia. In the study we determined the expression of metalloproteinase type 9 and its inhibitor (TIMP-1). We found sufficient expression of TIMP-1 in the peripheral cytotrophoblast, villous and syncytial buds, which are washed by maternal blood. This explains the presence of paracrine regulation, restrictive cytotrophoblast invasion in the last months of pregnancy. For the first time we have obtained evidence of increased expression of tissue inhibitors of metalloproteinases (in particular TIMP-1) and the weakening of the expression of their substrates (MMPs) in severe preeclampsia by invasive cells, but not in placental tissue homogenates or invasive cells in culture (as previously shown in other studies).
Key words: immunohistochemistry, TIMP-1, MMP-9, severe pre-eclampsia

Было проведено гистохимическое исследование плацент здоровых беременных и беременных с тяжелой поздней преэклампсией. В ходе исследования определялась экспрессия металлопротеиназы 9 типа и ее ингибитора (TIMP-1). Обнаружена значимая экспрессия TIMP-1 в периферическом цитотрофобласте клеточных островков, ворсин и синцитиальных почках, которые омываются материнской кровью. Это объясняет наличие паракринной регуляции, сдерживающей цитотрофобластическую инвазию в последние месяцы беременности. Впервые получены данные об усилении экспрессии тканевых ингибиторов металлопротеиназ (в частности TIMP-1) и ослаблении образования их субстратов (MMPs) при тяжелой преэклампсии инвазивными клетками, а не в гомогенатах плацентарной ткани, либо в культуре инвазивных клеток (как было показано ранее в других исследованиях).
Ключевые слова: иммуногистохимия, TIMP-1, MMP-9, тяжелая преэклампсия

Hypertensive complications are a major cause of maternal mortality in developing countries (11–16%) [3]. Women with a history of preeclampsia (PE) and their offspring are exposed in later life increased risk of cardiovascular and renal disease [2, 4, 8]. It is likely that the elucidation of etiologic and pathogenetic mechanisms of PE allows to find approaches to the treatment and prevention of its development. Numerous studies have confirmed the presence of two phenotypic variants of PE: early PE (before 34 weeks) and late PE (after 34 weeks) [6, 7]. Changes in the expression of a number of factors including matrix metalloproteinases (MMPs) leads to insufficient cytotrophoblast invasion and incomplete spiral artery remodeling. The key effectors of the remodeling of the extracellular matrix are matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1). These factors are the only proteolytic enzymes that are able to degrade collagen, a structural protein of basement membranes [5]. There are biological mechanisms to limit proteolysis tissue caused by active metalloproteinases (MMPs). Stromal cells secreted tissue inhibitors of metalloproteinases

(TIMPs), which are small in size, capable of forming non-covalent complexes with MMPs [1]. TIMP-1 is a tissue inhibitor of MMP-9.

Objective: evaluation of expression of MMP-9 and TIMP-1 in the basal lamina and placental villi of women with physiological term pregnancy and women with severe late PE.

Material and Methods. We studied samples of placentas of pregnant women (n=40) who were treated in the Stavropol Regional Clinical Perinatal Center. Patients were divided into 2 groups. The criteria for inclusion in the main group (n=20) were clinical manifestations of severe PE after 34 weeks, singleton pregnancy, absence of severe somatic and autoimmune diseases, informed consent of the patient. Pregnant women from the main group were given birth by caesarean section. The indications for surgery were negative dynamics of PE (11 cases), acute fetal hypoxia (5 cases), the combination of PE with burdened history (2 cases) and the combination of PE with a uterine scar (2 cases). The criteria for inclusion in the control group (n=20) were full-term physiological pregnancy, absence of diseases involving change of the angiogenic status, women without a history of PE in the previous pregnancy and informed consent of the patient. Pregnant women from the control group were given birth by caesarean section at relatively prosperous pregnancy. The indications for surgery were the large size of the fetus in the breech position (8 cases), mixed breech presentation in primiparous or foot fetal presentation (3 cases), oblique or transverse position of the fetus (5 cases), high-degree myopia with changes on an eyeground (4 cases).

Fragments of the placenta were received during cesarean section. We selected at least six fragments from each placenta. Preparations were stained with hematoxylin and eosin for preliminary review and further review of invading elements (microscope Leica DM2500). Serial sections were subjected to immunohistochemical examination according to standard protocols. Immunoreactivity of the primary antibodies was detected using secondary antibodies conjugated with peroxidase system. We used antibodies to detect cytokeratin 8 type (cytokeratin 8, Novocastra, clone TS1; detection system Thermo Scientific Ultravision Detection System AntiOlyvalent, HRP/DAB). On the remaining serial sections we used ready to use antibodies to detect MMP-9 (Novocastra, clone 15W2) and TIMP-1 (Novocastra, clone 6F6a). We have used a single detection system Thermo Scientific Ultravision Detection System Anti-Polyvalent in all cases. Using these markers we investigated the interstitial cytotrophoblast (ISC), separate multinucleated giant cells (MGC), the intermediate cytotrophoblast (IMC) and maternal decidual cells (DC). We evaluated the intensity of the expression of these cells at x200 magnification. The results of immunohistochemical reaction was assessed by semi-quantitative analysis of color intensity: when considering the TIMP-1 level for the negative level («–») has been accepted level of color surrounding the decidual cells, «+/–» – a minimal coloring, «+» – weak coloring, «++» – moderate coloring, «+++» – expressed intensity of the color. For MMP-9 level color surrounding decidual cells was adopted as a «+/–», as known from the literature that these cells are weakly positive for this marker [9]. Accordingly, «–» – negative coloring, «+» – the average intensity of the color, «++» – the maximum intensity of the color. In each case we examined 10 cells of each type.

Results and Discussion. The study found that at the full-term placenta takes place sufficient expression of MMP-9 in invasive cells (Table).

Table

Expression of MMP-9 and TIMP-1 in the cells of the placenta in normal and severe late preeclampsia (conditional points)

Separate cells	Normal pregnancy		Severe preeclampsia	
	MMP-9	TIMP-1	MMP-9	TIMP-1
ISC	+	+	+	+
MGC	+	–	+	+
IMC	–	+	+	++
SB	–	+	–	–
DC	+/–	+/–	+	+/–

ISC – interstitial cytotrophoblast, MGC – separate multinucleated giant cells, IMC – intermediate cytotrophoblast, SB – syncytial buds, DC – maternal decidual cells.

Despite the completion of cytotrophoblast invasion activity at the end of pregnancy the expression of MMP-9 conserved in the surface layer of ISC, under Rohr's fibrinoid layer and in invasive cells which penetrate into the decidua (Fig. 1a).

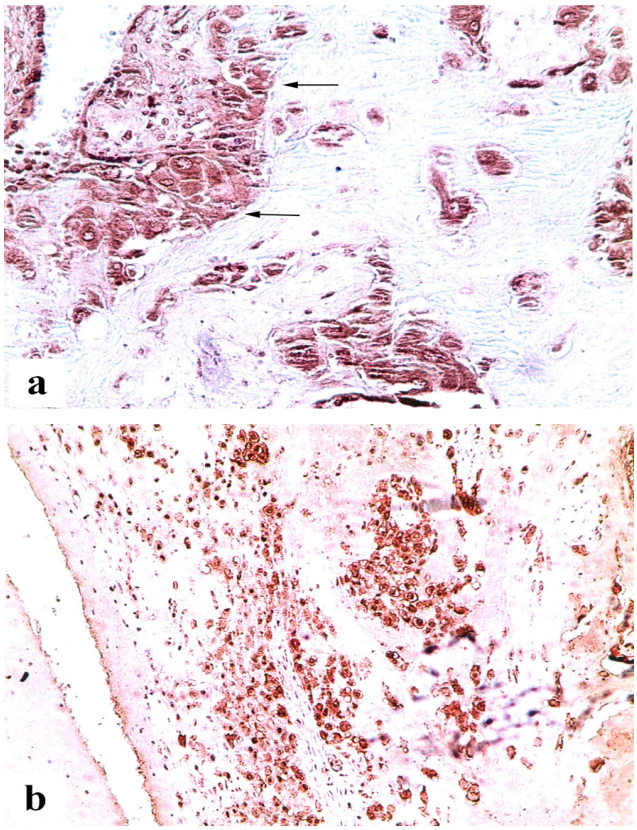

Fig. 1. The placenta in normal full-term pregnancy, visualization MMP-9 and TIMP-1 in the basal plate: a) a group of ISC (indicated by arrows) expressing MMP-9 (++) in the surface layer, x400; b) ISC expressing TIMP-1 (+) x100

This corresponds to a similar degree of expression of TIMP-1 in ISC (Fig. 1b). An important addition is to reveal the expression of TIMP-1 in peripheral cytotrophoblast and a weakly positive reaction in the surrounding villous epithelium (Fig.1b). Positive expression of TIMP-1 is noted in the epithelium of the villi and syncytial buds and negative or indeterminate expression 1 is noted in the decidual cells.

Thus the final stage of cytotrophoblast invasion in the placenta of healthy women is characterized by a particular reaction to MMP-9 and TIMP-1 in invasive cells, which is a consequence of an autocrine restrictive regulation of the activity of these factors. We found sufficient expression of TIMP-1 in the peripheral cytotrophoblast, villous and syncytial buds, which are washed by maternal blood. Considering the large area of covering syncytial villous tree in the maternal bloodstream should do enough TIMP-1, which indirectly restrains cytotrophoblast invasion in the last months of pregnancy (paracrine regulation).

MMP-9 and TIMP-1 in the study showed of placentas in preeclampsia contrast change when compared to gestational norm (Table 1). All species of invasive trophoblast (ISC, MGC, IMC) showed quantitative and qualitative changes.

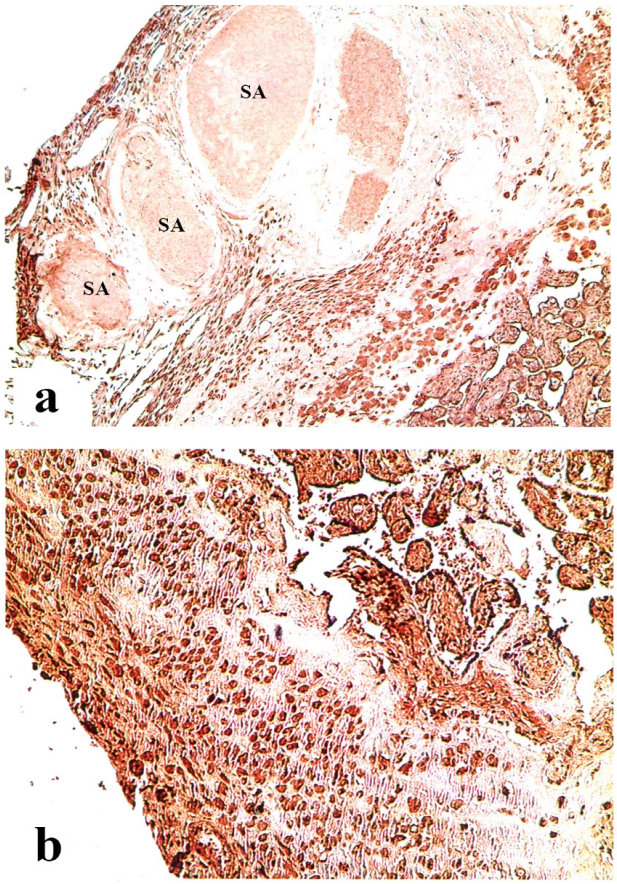

Fig. 2. The placenta in severe pre-eclampsia: a) around wide spiral arteries (SA) bundles of narrow, small invasive cells, x50; b) a fragment of: various shapes of ISC, the longitudinal – closer to the villi and cross – to the left at a sufficient expression of MMP-9 in all invasive cells, x200

Expression of MMP-9 in ISC emphasizes small sizes of the cells having an elongated or oval shape depending on the slice (Fig. 2). In mounts we found wide endometrial spiral arteries, which confirms the usefulness of the first wave of cytotrophoblast invasion (Fig. 2a). However, IMC rarely penetrates into the deeper layers of the basal plate of the placenta, cells change direction, form a multi-directional bundles (Fig. 2b), which is likely to reduce their invasive activity.

The study has been convincingly shown TIMP-1 expression in the intermediate cytotrophoblast (Fig. 3).

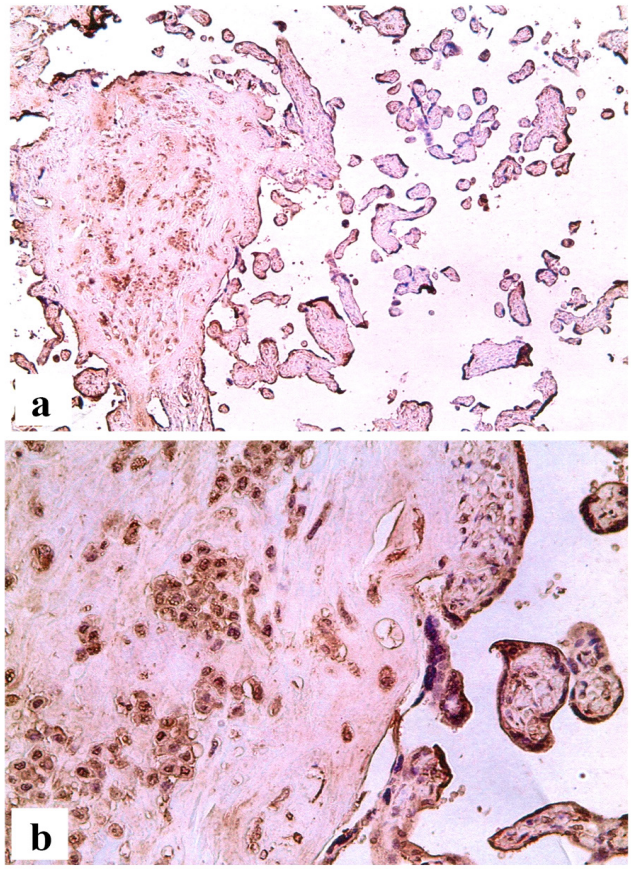

Fig. 3. The placenta in severe pre-eclampsia: a) islet cell: sufficient expression of TIMP-1 in the IMC, x50; b) a fragment: moderate expression (++) of TIMP-1 in the IMC, x200

Conclusions. The data on the expression of factors that play a leading role in the pathogenesis of PE confirming previous results on the increase in the expression of tissue inhibitor of metalloproteinases (in particular TIMP-1) and the weakening of the expression of their substrates (MMPs) in severe PE in placental tissue homogenates or invasive cell culture. Despite the fact that the main events that result in a PE occur even at 12-14 weeks of pregnancy, in the third trimester detected changes in the system «protease-antiprotease» with severe PE compared to the norm. This confirms the continuation throughout pregnancy pathogenic factor which is still at the beginning of gestation points to the impossibility of strengthening implantation.

References

1. Birkedal-Hansen H., Moore W. G., Bodden M. K., Windsor L.J., Birkedal-Hansen B., DeCarlo A., Engler J. A. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med* 1993;4(2):197-250.
2. Davis E.F., Lazdam M., Lewandowski A. J., Worton S. A., Kelly B., Kenworthy Y. et al. Cardiovascular risk factors in children and young adults born to preeclamptic pregnancies: a systematic review. *Pediatrics* 2012;129(6):e1552–61. doi:10.1542/peds.2011-3093
3. Khan K. S., Wojdyla D., Say L., Gülmezoglu A. M., Van Look P. F. WHO analysis of causes of maternal death: a systematic review. *Lancet* 2006;367(9516):1066-1074. doi:10.1016/S0140-6736(06)68397-9
4. McDonald S. D., Malinowski A., Zhou Q., Yusuf S., Devereaux P. J. Cardiovascular sequelae of preeclampsia/eclampsia: a systematic review and meta-analyses. *Am. Heart J* 2008;156(5):918-930. doi:10.1016/j.ahj.2008.06.042
5. Mutter W. P., Karumanchi S. A. Molecular mechanisms of preeclampsia. *Microvasc. Res.* 2008;75(1):1-8.
6. Nelson D. B., Ziadie M. S., McIntire D. D., Rogers B. B., Leveno K. J. Placental pathology suggesting that preeclampsia is more than one disease. *Am. J. Obstet. Gynecol*. 2014; 210(1):66:e1-7. doi:10.1016/j.ajog.2013.09.010
7. Peraçoli J. C., Bannwart-Castro C. F., Romao M., Weel I. C., Ribeiro V. R., Borges V. T. High levels of heat shock protein 70 are associated with pro-inflammatory cytokines and may differentiate early- from late-onset preeclampsia. *J. Reprod. Immunol*. 2013;100(2): 129-34. doi:10.1016/j.jri.2013.08.003
8. Vikse B. E., Irgens L. M., Leivestad T., Skjaerven R., Iversen B. M. Preeclampsia and the risk of end-stage renal disease. *N. Engl. J. Med.* 2008;359(8):800-809. doi:10.1056/NEJMoa0706790
9. Xu P., Alfaidy N., Challis J. R. Expression of matrix metalloproteinase (MMP)-2 and MMP-9 in human placenta and fetal membranes in relation to preterm and term labor. *J Clin Endocrinol Metab*. 2002;87(3):1353-61.

About authors:

Milovanov Andrey Petrovich, MD, PhD, Professor, Head of the Laboratory for pathology of the female reproductive system, Research Institute of Human Morphology, Moscow, Russia; tel.: +74991295501; e-mail: a_p_milovanov@mail.ru

Dubovoy Alexander Alexandrovich, Assistant of obstetrics and gynecology department, Stavropol State Medical University, Russia; tel.: +79288100109; e-mail: alexanderdubovoy@gmail.com

Aksenenko Victor Alekseevich, MD, PhD, Professor, Head of the obstetrics and gynecology department, Stavropol State Medical University, Russia; tel.: +78652716536; e-mail: aksenenko_@mail.ru

Rasstrigina Irina Mikhailovna, Research associate of the Laboratory for pathology of the female reproductive system, Research Institute of Human Morphology, Moscow, Russia; tel.: +79629330270; e-mail: runa.77@mail.ru

Fokina Tatyana Vasilyevna, Senior researcher of the Laboratory for pathology of the female reproductive system, Research Institute of Human Morphology, Moscow, Russia; tel.: +74991295501

Nikolskaya Tatyana Nikolaevna, Assistant of obstetrics and gynecology department, Stavropol State Medical University, Russia; tel.: +79624065982

UDC – [618.3-06: 616.314.17-002]-092: 612.017.1-00864]-07-08(045)
DOI – http://dx.doi.org/10.14300/mnnc.2015.10034
ISSN – 2073-8137

SPECIFIC FEATURES OF PERIPHERAL BLOOD LYMPHOCYTES IMMUNOPHENOTYPE IN PREGNANT WOMEN WITH CHRONIC PERIODONTITIS

Prohodnaya V. A.

Rostov State Medical University, Rostov-on-Don, Russian Federation

ОСОБЕННОСТИ ИММУНОФЕНОТИПА ЛИМФОЦИТОВ ПЕРИФЕРИЧЕСКОЙ КРОВИ У БЕРЕМЕННЫХ ЖЕНЩИН С ХРОНИЧЕСКИМ ПАРОДОНТИТОМ

В. А. Проходная

Ростовский государственный медицинский университет, Ростов-на-Дону, Российская Федерация

Purpose of study was to identify the specific features of cellular immunity through immunophenotypic analysis of peripheral blood lymphocytes in pregnant women with inflammatory periodontal disease depending on the structure of the pathology as well as the severity of chronic periodontitis. 165 pregnant women with inflammatory periodontal disease; 31 pregnant women with physiological pregnancy; 32 healthy female volunteers. All the women had their immunophenotype of peripheral blood lymphocytes investigated. The immunophenotyping of the lymphocytes bearing the $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, and $CD95^+$ markers was performed with laser flow cytometry. The study showed that along with an increasing degree of chronic periodontitis severity the pregnant women developed more pronounced lymphopenia, imbalance in T cell subsets with a decrease in the relative number of mature T-lymphocytes and T-helper cells against an increasing number of cells possessing killer and apoptotic activity. The pregnant women with chronic periodontitis revealed changes in the lym-