

DOI: 10.26693/jmbs03.07.112

UDC 618.39-021.3-078:577.151.042.083.185

Korovay S. V., Stetsenko S. O., Bondareva A. V.

THE «PROTEINASES – INHIBITORS OF PROTEINASES» SYSTEM'S ACTIVITY IN WOMEN WITH PREVENTION OF PREGNANCY IN THE EARLY AND LONG TERM

Kharkiv Medical Academy of Postgraduate Education, Department of Obstetrics,
Perinatology and Gynecology, Ukraine

Korovai.sergei@gmail.com

Despite numerous studies, the pathogenesis of the premature abortion development is not fully disclosed. There is also no generally accepted theory that would explain the whole variety of violations and allowed to substantiate the effective complex of treatment and preventive measures for the prevention of reproductive losses and the obstetric complications remote development.

The purpose of the study was to study the features of the «proteinase – inhibitor proteinase» system state by detecting the activity of neutrophil elastase and plasma concentrations of proteinase inhibitors α 1-antitrypsin and α 2-macroglobulin in women with different periods of pregnancy termination.

Material and methods. 227 pregnant women who were admitted to the Kharkiv city perinatal center were examined, 190 of them had clinical signs of premature pregnancy termination in the period of gestation of 23–36 weeks. Formation of clinical groups was carried out depending on the term of pregnancy in the form of premature and timely birth.

The criteria for including women into groups were the following: young reproductive age, single-pregnancy, absence of gestosis, acute and chronic gynecological and somatic diseases. Detection of preterm labor was carried out in the presence of abdominal pain syndrome and structural changes in the cervix.

Conclusions. The disturbance of the dynamic equilibrium between the activity of endogenous proteolytic enzymes and the content of their inhibitors, which is confirmed by the reliable ($p \leq 0.001$) increase in the activity of neutrophil elastase, by an average of 46%, together with a 36% increase in plasma α 1-antitrypsin plasma levels and a decrease in the concentration of α 2-macroglobulin on 30% was observed in women with preterm labor in the period of 23–27 weeks in relation to women with the physiological course of pregnancy, which ended with childbirth without complications in the period of 38–41 weeks. The change in the rates was less pronounce in women with preterm labor in the period of 28–36 weeks in

relation to women in the control group: an increase in neutrophil elastase was an average of 25% ($p < 0.001$) and plasma concentration α 1-antitrypsin 21% ($p = 0.006$) with a decrease concentration of α 2-macroglobulin by 13% ($p = 0.142$).

The tension of proteolytic processes was most pronounced in the direction of the proteinase activity prevalence in women with preterm labor in the period of 26–30 weeks, which was confirmed by the probable ($p \leq 0.014$) increase in the ratio of the activity of neutrophil elastase to the sum of concentrations of inhibitors of α 1-AT and α 2-MG on average 32%.

The imbalance in the «proteinase – inhibitors of proteinase» system is one of the links in the pathogenesis of non-pregnancy and can contribute in combination with other complications.

Keywords: premature birth, proteinase, neutrophil elastase, proteinase inhibitors.

Research relation to the plans, programs and department themes. The study was carried out within the framework of the department research: «Features of treatment and prevention of pathological conditions in the pregravidder period of gestation and ways of their correction» (state registration number is 0111U003583).

Introduction. Despite numerous studies, the pathogenesis of the premature abortion development is not fully disclosed. There is also no generally accepted theory that would explain the whole variety of violations and allowed to substantiate the effective complex of treatment and preventive measures for the prevention of reproductive losses and the obstetric complications remote development [11]. This, in turn, stimulates scientists to study the etiopathogenesis of preterm labor in women more deeply to find more rational pathogenetic's treatment. A significant number of factors affect the course of pregnancy and its outcome. Their action is realized at the final stage through the homeostatic systems of the organism, where an important role is assigned to the proteinase-

proteinase inhibitors system [3]. Elastase is a strong proteolytic enzyme with a broad spectrum of activity, properties of a inflammation mediator, a stimulator of metabolic processes, a factor of permeability and others [8]. Scientists are paying attention to neutrophilic elastase in recent times. Its diagnostic value for many pathological processes is at the stage of study. It should be noted that during certain periods of pregnancy the increase of immune system cells functional activity occurs, in particular neutrophils, in order to compensate for the depression of the adaptive immune system cells of the parent organism [7]. It is proved that neutrophilic elastase is involved in the remodeling of the uterus tissues during the period of the invasive cytotrophoblast growth, as well as in the labor activity induction and maintenance [7]. But uncontrolled proteolysis can lead to negative consequences for the body, cause destruction of the extracellular matrix components of tissues and vessels, promote the formation of vasoactive compounds, therefore its blood level is regulated by a complex system of protein-inhibitors [5]. Among the main inhibitors of proteolytic enzymes are α 1-antitrypsin (α 1-AT) and α 2-macroglobulin (α 2-MG), the level determination of which is carried out in order to characterize the course and prognosis of various pathological conditions [2,10]. The lack of literature information on the system state «proteinase-inhibitor proteinase» in women with a threat of preterm labor stimulates the importance for its study and justification of the relationship with other etiological pathogenesis factors.

The purpose of the study was to study the features of the «proteinase – inhibitor proteinase» system state by detecting the activity of neutrophil elastase and plasma concentrations of proteinase inhibitors α 1-antitrypsin and α 2-macroglobulin in women with different periods of pregnancy termination.

Material and methods of research. 227 pregnant women who were admitted to the Kharkiv city perinatal center were examined, 190 of them had clinical signs of premature pregnancy termination in the period of gestation of 23–36 weeks. Formation of clinical groups was carried out depending on the term of pregnancy in the form of premature and timely birth. 48 women with early pregnancy termination (mean age 23.6 ± 5.1 years) were included in group I, which ended in childbirth in the period from 23 to 27 weeks. All pregnant women with a risk of premature birth, depending on the gestational period, had the following distribution: Ia subgroup included 23 pregnant women at 23–25 weeks; Ib subgroup included 25 pregnant women at 26–27 weeks. In group II 142 women (mean age 24.7 ± 4.2 years) with late term of preterm pregnancy in the period from 28 to 36 weeks were involved, depending on the term of birth. These

patients were divided into subgroups: IIa – 38 pregnant women at 28–30 weeks; IIb – 48 pregnant women at 31–33 weeks; IIc – 56 pregnant women at 34–36 weeks. The III (control) group included 37 women with a physiological course of pregnancy (mean age 26.1 ± 2.7 years), which ended in childbirth without complications at the time of 38–41 weeks. The criteria for including women into groups were: young reproductive age, single-pregnancy, absence of gestosis, acute and chronic gynecological and somatic diseases. Detection of preterm labor was carried out in the presence of abdominal pain syndrome and structural changes in the cervix. The research was carried out in accordance with the bioethics principles.

The activity of neutrophilic elastase was determined by the immunoassay method using Human Elastase ELISA diagnostic test systems (Germany) and the StatFAQ 303 Rlus immunoassay analyzer. Determination of the concentrations of α 1-AT and α 2-MG in blood plasma was performed by a complex method using the N-benzoyl-L-arginine-paranitro-anilide substrate [4]. The statistical processing of the obtained results was carried out using licensed standardized packages of multivariate statistical analysis Statistica 6.1. applications. Median sampling (Me), the bottom (Q25) and upper (Q75) quartiles were used, taking into account the lack of normal data distribution. Comparison of the indices in the groups was carried out using the non-parametric Mann-Whitney test.

Results and discussion. We determined an increase in the activity of neutrophil elastase on average by 46% in women of group I, (in the Ia subgroup by 42%, Ib in the subgroup by 49%) in relation to the control group women ($p < 0.001$) (**Table 1**). It should be noted that a probable ($p = 0.003$) increase in enzyme activity when compared with group II by 15%. 28 patients (58%) of group I had elastase activity beyond the control range. In particular, in the Ia subgroup this was observed in 13 cases (57%), and in Ib subgroup – in 15 cases (60%). The activity of neutrophil elastase was also significantly increased ($p < 0.001$) in women with preterm labor in the gestation period of 28–36 weeks (group II) in relation to women with physiological pregnancy (group III), but less pronounced – an average of 25%. The increase in the activity of the enzyme in relation to the control group was accordingly 31% ($p < 0.001$) in the IIa subgroup, and 25% ($p < 0.001$) in the IIb subgroup, and – 20% ($p = 0.0042$) in the IIc subgroup. Excess of the upper range of control was noted in 50 cases (35%) in this group of patients.

The results showed that in the blood plasma of women with preterm labor in the period of 23–27 weeks (Group I), with the activation of neutrophil

Table 1 – Dynamics of the changes «proteinase - inhibitor proteinase» parameters in the blood plasma of the observation groups (Me (Q25; Q75))

Indicator	I group (23–27), n = 48		II group (28–36), n = 142			III group (38–41) n = 37
	Ia (23–25) n = 23	Ib (26–27) n = 25	Ila (28–30) n = 38	Ilb (31–33), n = 48	IIV (34–36) n = 56	
Elastase, ng/ml	88,6 (77,3; 97,2) ¹		77,5 (58,3; 90,3) ^{1,2}			56,9 (49,3; 67,4)
	88,3 (76,4; 97,2) ¹	90,1 (79,5; 96,3) ¹	79,6 (60,4; 90,5) ¹	76,4 (56,6; 89,7) ¹	68,8 (55,2; 89,1) ¹	
Alpha-1-antitrypsin, mcM/(s·l)	18,4 (15,5; 21,9) ¹		17,0 (12,2; 19,6) ²			13,5 (10,3; 17,4)
	19,5 (17,3; 22,2) ¹	17,3 (14,9; 21,0) ¹	14,2 (9,82; 17,4)	17,7 (14,3; 23,5) ¹	17,1 (13,4; 19,5) ¹	
Alpha-2-macroglobulin, mcM/(s·l)	2,1 (1,8; 2,7) ¹		2,7 (2,0; 3,1) ²			2,9 (1,9; 3,9)
	1,9 (1,5; 2,4) ¹	2,3 (1,8; 2,8) ¹	3,3 (2,6; 3,8)	2,2 (1,8; 2,8) ¹	2,7 (2,1; 3,0)	
Elastase / α1-AL+α2-MG, s.u.	4,13 (3,37; 5,22) ¹		3,79 (2,95; 5,04)			3,40 (2,81; 4,54)
	4,02(3,02; 5,16)	4,25 (3,82; 5,72) ¹	4,49 (3,22; 6,40) ¹	3,65 (2,72; 4,67)	3,64 (2,75; 4,79) ¹	

Note: 1 – differences from group III are statistically significant at the level $p < 0,05$ – $0,001$; 2 – the differences between I and II groups are statistically significant at the level $p < 0,05$ – $0,001$.

count, activation of neutrophil count, a ($p \leq 0.001$) increase in the average concentration of $\alpha 1$ -AT by 36% was observed with a decrease in the concentration of $\alpha 2$ -MG by 30% in comparison with women of group III (**Table 1**). A similar trend was observed in comparison with the II group. There was an increase ($p = 0,023$) by 14% of $\alpha 1$ -AT level and a decrease ($p < 0,001$) by 19% of $\alpha 2$ -MG levels. The content was beyond the range of control group values for $\alpha 1$ -AT in 16 (33%) group I, and for $\alpha 2$ -MG it was observed only in 16 cases (11%). The most pronounced increase in $\alpha 1$ -AT levels ($p < 0.001$) and $\alpha 2$ -MH levels ($p = 0.0021$) in control group in preterm labor in the period of 23–25 weeks (Ia subgroup) was decreased by an average of 43% and 33%, whereas in the period of 26–27 weeks, respectively, 26% ($p = 0.003$) and 24% ($p = 0.0225$).

$\alpha 1$ -AT concentration was also significantly increased ($p = 0.0059$) by 21% in the blood plasma of group II in relation to women with physiologic gestational age, whose pregnancy ended without complications, but for $\alpha 2$ -MG, it was unlikely to be recorded ($p = 0.148$) decrease by an average of 13% (**Table 1**). The concentration of $\alpha 1$ -AT was above the control range of 32 patients in group II (23%), whereas in the case of $\alpha 2$ -MH, this was only characteristic of 9 subjects (6%). The probable increase (≤ 0.006) of $\alpha 1$ -AT in subgroups was observed in women whose pregnancy ended in childbirth at the time of 31–33 weeks and 34–36 weeks on average by 34 and 20% respectively, whereas the level the indicator was almost equal to control ($p = 0.698$) in the period of 28–30 weeks. Another picture of the changes is characteristic of $\alpha 2$ -MG: it is probable ($p = 0.0084$) in relation to the control of a decrease of 27% in women IIb sub-

groups at a practically unchanged level in women IIb and IIv subgroups ($p = 0.458$ and $p = 0.146$, respectively).

Consequently, the disturbance of the dynamic equilibrium in the «proteinase – inhibitors of proteinase» system occurs in women with preterm labor, especially in the early stages, which is confirmed by the marked increase in the activity of neutrophilic elastase. On the other hand, elastase activity occurs on the background of a decrease of the proteinase inhibitor $\alpha 2$ -MG concentration and increase of $\alpha 1$ -AT concentration, which is probably insufficient to inactivate high proteolytic activity. It should be noted that the decrease in the level of $\alpha 2$ -MG in female groups leads not only to the disturbance of the balance in the «proteinase – inhibitors of proteinase» system in the direction of proteinase activity, but also to the disorder of the mechanisms in which this protein is involved, in particular immunosuppression in pregnancy [1].

The tension of proteolytic processes in women with preterm labor in different terms was also judged by the ratio of the neutrophil elastase activity to the sum of inhibitors concentrations $\alpha 1$ -AT and $\alpha 2$ -MG (**Table 1**). The results showed a reliable ($p = 0.0144$) in relation to the control of its increase in the group I of women on average by 19%, especially due to the value of Ib in the subgroup – by 31% ($p = 0.0025$), whereas in Ia this sub-group was unlikely ($p = 0.319$) and was only 9%. The value of the correlation coefficient was unlikely ($p = 0.215$) for women of group II, in relation to women of group III, but in the Ila subgroup, a significant ($p = 0.014$) increase of the coefficient was registered at 32%, while in subgroups IIb and IIc the values were almost equal to control. These results also confirm the proteinase activity prevalence,

especially in women with preterm labor in the period of 26–30 weeks (Ib and IIa subgroups). Increased activity of neutrophil elastase is a prognostic sign in the opinion of some authors, indicating the danger of premature births [6, 9]. Increased proteolytic activity can also be a factor in the violation of plasma proteolytic system regulatory mechanisms, responsible for adaptive processes [8].

Conclusions

1. The disturbance of the dynamic equilibrium between the activity of endogenous proteolytic enzymes and the content of their inhibitors, which is confirmed by the reliable ($p \leq 0.001$) increase in the activity of neutrophil elastase, by an average of 46%, against a background of a 36% increase in plasma α_1 -antitrypsin plasma levels and a decrease in the concentration of α_2 -macroglobulin on 30% was observed in women with preterm labor in the period of 23–27 weeks in relation to women with the physiological course of pregnancy, which ended with childbirth without complications in the period of 38–41 weeks. The change in the rates was less

pronounced in women with preterm labor in the period of 28–36 weeks in relation to women in the control group: an increase in neutrophil elastase is an average of 25% ($p < 0.001$) and plasma concentration α_1 -antitrypsin 21% ($p = 0.006$) with a decrease concentration of α_2 -macroglobulin by 13% ($p = 0.142$).

2. The tension of proteolytic processes is most pronounced in the direction of the proteinase activity prevalence in women with preterm labor in the period of 26–30 weeks, which is confirmed by the probable ($p \leq 0.014$) increase in the ratio of the activity of neutrophil elastase to the sum of concentrations of inhibitors of α_1 -AT and α_2 -MG on average 32%.
3. The imbalance in the «proteinase – inhibitors of proteinase» system is one of the links in the pathogenesis of non-pregnancy and can contribute in combination with other complications.

Prospects for further research. In the future, the authors plan to continue studying the state of the pathogenesis of the development of premature abortion.

References

1. Bazhenova LG. Biologicheskie svojstva belkov semejstva makroglobulinov i ih rol' v neoplasticheskom processe v jaichnikah. *Ross vestnik akushera-ginekologa*. 2011; 11(1): 17–21. [Russian]
2. Divocha VA, Deribon EL. Rol' ingibitorov proteinaz v patogeneze zabolevanij cheloveka. *Aktual'nye problemy transportnoj mediciny*. 2013; 2(32): 127–37. [Russian]
3. Fomochkina II, Kubyskin AV. Patogeneticheskoe znachenie proteinazno-ingibitornoj sistemy v razviti lokal'noj i sistemoj patologii. *Patologija*. 2012; 2(25): 50–4. [Russian]
4. Karjagina IJu, Zaremskij RA, Baljabina MD. Ispol'zovanie metoda kompleksnogo opredelenija aktivnosti tripsinopodobnyh proteinaz, α_1 -antitripsina i α_2 -makroglobulina v gastroenterologicheskoy klinike. *Laboratornoe delo*. 1990; 2: 10–3. [Russian]
5. Mihal'chuk VN. Tripsinopodobnye proteinazy v fiziologii i biologii cheloveka. *Aktual'nye problemy transportnoj mediciny*. 2008; 4(14): 134–9. [Russian]
6. Nakai A, Y Taniuchi, Miyake H, Nakai M, Yokota A, Takeshita T. Increased level of granulocyte elastase in cervical secretion is an independent predictive factor for preterm delivery. *Gynecol Obstet Invest*. 2005; 60(2): 87–91. PMID: 15809507. DOI: 10.1159/000084839
7. Nekrasova IV, Shirshov SV. Zhenskije polovye steroidnye gormony v reguljacii fermentativnoj aktivnosti nejtrofilov. *Doklady akademii nauk*. 2013; 453(6): 690–3. [Russian]
8. Paramonova NS, Ed. *Sostojanie jelastaza-ingibitornoj sistemy u detej v norme i pri otdel'nyh patologicheskikh sostojanijah*. Grodno: GrGMU; 2017. 132 p. [Russian]
9. Savchenko TN, et al. Citokiny i nejtrofil'naja jelastaza pri nevyshivanii beremennosti i genital'nom kandidoze. *Ros med zhurn*. 2009; 3: 20–3. [Russian]
10. Veremeenko KN. Ingibitory proteoliza – zashhitnye belki krovi. *Vrachebnoe delo*. 1987; 5: 45–8. [Russian]
11. Vorobiova II, Zhyvetska-Denysova AA, Tkachenko VB, ta in. Nevynoshuvnnia vahitnosti: suchasni pohliady na problemu (ohliad literatury). *Zdorove zhenshchyny*. 2017; 3(119): 113–7. [Ukrainian]

УДК 618.39-021.3-078:577.151.042.083.185

АКТИВНІСТЬ СИСТЕМИ «ПРОТЕІНАЗИ-ІНГІБІТОРИ ПРОТЕІНАЗ»

У ЖІНОК З ПЕРЕРИВАННЯМ ВАГІТНОСТІ В РАННІ ТА ПІЗНІ ТЕРМІНИ

Коровай С. В., Стеценко С. О., Бондарева А. В.

Резюме. Незважаючи на численні дослідження, патогенез розвитку передчасного переривання вагітності до кінця не розкритий, відсутня теорія, яка б дозволила обґрунтувати ефективний комплекс лікувально-профілактичних заходів при віддаленому розвитку акушерських ускладнень.

Мета дослідження – вивчити особливості стану системи «протеїнази-інгібітори протеїназ» шляхом визначення активності нейтрофільної еластази та плазмових концентрацій протеїназних інгібіторів у жінок з різними термінами переривання вагітності.

Обстежено 227 вагітних жінок, які поступили до Харківського міського перинатального центру, з них 190 мали клінічні ознаки загрози передчасних пологів.

У жінок з передчасними пологами в термін 23–27 тижнів відмічається порушення рівноваги між активністю ендогенних протеолітичних ензимів і вмістом їх інгібіторів, що підтверджується підвищенням активності нейтрофільної еластази в середньому на 46%, та зниження концентрації $\alpha 2$ -макроглобуліну на 30%. У жінок з перериванням вагітності в термін 28–36 тижнів зміна показників менш виражена: підвищення нейтрофільної еластази становить в середньому 25%, та плазмової концентрації $\alpha 1$ -антитрипсину – 21%. Найбільш виражена напруженість протеолітичних процесів виявлена у жінок з передчасними пологами у термін 26–30 тижнів, що підтверджується підвищенням коефіцієнта співвідношення активності нейтрофільної еластази до суми концентрацій інгібіторів $\alpha 1$ -АТ і $\alpha 2$ -МГ у середньому на 32%.

Дисбаланс у системі «протеїнази-інгібітори протеїназ» складає одну із ланок патогенезу невиношування вагітності, й може сприяти у комплексі з іншими розвитку ускладнень.

Ключові слова: невиношування вагітності, протеїназа, нейтрофільна еластаза, протеїназні інгібітори.

УДК 618.39-021.3-078:577.151.042.083.185

АКТИВНОСТЬ СИСТЕМЫ «ПРОТЕИНАЗЫ-ИНГИБИТОРЫ ПРОТЕИНАЗ»

У ЖЕНЩИН С ПРЕРЫВАНИЕ БЕРЕМЕННОСТИ В РАННИЕ И ПОЗДНИЕ СРОКИ

Коровай С. В., Стеценко С. А., Бондарева А. В.

Резюме. Несмотря на многочисленные исследования, патогенез развития преждевременного прерывания беременности до конца не раскрыт, отсутствует теория, которая бы позволила обосновать эффективный комплекс лечебно-профилактических мероприятий при отдаленном развитии акушерских осложнений.

Цель исследования – изучить особенности состояния системы «протеиназы-ингибиторы протеиназ» путем определения активности нейтрофильной эластазы и плазменных концентраций протеиназ ингибиторов у женщин с различными сроками прерывания беременности.

Обследовано 227 беременных женщин, поступивших в Харьковский городской перинатальный центр, из них 190 имели клинические признаки угрозы преждевременных родов.

У женщин с преждевременными родами в срок 23–27 недель отмечается нарушение равновесия между активностью эндогенных протеолитических энзимов и содержанием их ингибиторов, что подтверждается повышением активности нейтрофильной эластазы в среднем на 46%, и снижение концентрации $\alpha 2$ -макроглобулина на 30%. У женщин с прерыванием беременности в срок 28–36 недель изменение показателей менее выражено: повышение нейтрофильной эластазы составляет в среднем 25%, и плазменной концентрации $\alpha 1$ -антитрипсина – 21%. Наиболее выраженная напряженность протеолитических процессов определена у женщин с преждевременными родами в срок 26–30 недель, что подтверждается повышением коэффициента соотношения активности нейтрофильной эластазы к сумме концентраций ингибиторов $\alpha 1$ -АТ и $\alpha 2$ -МГ в среднем на 32%.

Дисбаланс в системе «протеиназы-ингибиторы протеиназ» составляет одну из звеньев патогенеза невынашивания беременности, и может способствовать в комплексе с другими развитию осложнений.

Ключевые слова: невынашивание беременности, протеиназа, нейтрофильная эластаза, протеиназные ингибиторы.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of coauthors of the article.

Стаття надійшла 01.09.2018 р.

Рекомендована до друку на засіданні редакційної колегії після рецензування