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DIAGNOSTIC IMPORTANCE OF METALLOPROTEINASE ACTIVITY IN THE PREGRAVID PERIOD AND DURING PREGNANCY IN WOMEN WITH A HISTORY OF HEPATOBILIARY DISEASES

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The aim of study. An increase in the concentration of matrix metalloproteinase type 9 (MMP-9) and tissue inhibitor of metalloproteinase type 1 (TIMP-1) in the blood serum during pregnancy in women with a burdened premorbid background indicates their role at the beginning of the placentation process. A significant increase in tissue proteolysis enzyme (MMP-9) with a decrease in the concentration of TIMP-1 in the blood of women with a threat of miscarriage was established, which can be considered a sign of impaired homeostasis of the extracellular matrix.

Materials and methods. In the pregravid period the patients with impaired liver function (non-alcohol steatohepatitis (NASH), liver steatosis) have intensification of lipid peroxidation (an increase of the metabolites in the blood) and insufficiency in the glutathione system, which determines the profound destructive processes at the systemic level. A violation of the lipid spectrum of the blood in the form of hypercholesterolemia and the growth of its atherogenic fractions was observed in the examined patients.

Results. An increase of the MMP-9 level in the blood serum was found in chronic hepatobiliary diseases, and its highest level was detected with exacerbation, which indicated the inflammatory process activation and transformation of steatosis into fibrosis. The concentration of the tissue inhibitor TIMP-1 correlated with the severity of the inflammatory process – the increase in its level is more with NASH than with hepatic steatosis.

Key words: pregravid period, miscarriage, liver steatosis, non-alcohol hepatitis, metalloproteinase type 9, inhibitor of metalloproteinase type 1.

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ДІАГНОСТИЧНЕ ЗНАЧЕННЯ АКТИВНОСТІ МЕТАЛОПРОТЕЇНАЗ У ПРЕГРАВІДАРНИЙ ПЕРІОД І ПІД ЧАС ВАГІТНОСТІ У ЖІНОК ІЗ ЗАХВОРЮВАННЯМИ ГЕПАТОБІЛІАРНОЇ СИСТЕМИ В АНАМНЕЗІ

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Встановлено суттєве зростання ферменту тканинного протеолізу за ускладненого перебігу вагітності порівняно з неускладненою вагітністю, що можна вважати ознакою порушення гомеостазу екстрацелюлярного матриксу. За сприятливого перебігу I триместру гестації встановлено збільшення рівня інгібітора TIMP-1 у сироватці крові, що розцінено як інгібіція клітин трофобласта. За ускладненого перебігу вагітності активність TIMP-1 зменшувалася. Виявлено зростання вмісту MMP-9 у сироватці крові, причому ступінь збільшення її концентрації залежав від вираженості патологічного процесу в печінці. Максимально високий рівень MMP-9 було виявлено за неалкогольного стеатогепатиту, що свідчило про активацію запального процесу та трансформацію стеатозу у фіброз. Концентрація тканинного інгібітора TIMP-1 корелювала з вираженістю запального процесу зростання його рівня за неалкогольного стеатогепатиту більше, ніж за стеатозу печінки.

Ключові слова: невиношування, стеатоз, стеатогепатит, матриксна металопротеїназа 9-го типу, тканинний інгібітор металопротеїназ 1-го типу.

Introduction. A common complication of early pregnancy is its termination. According to research [1], 13.5% of pregnancies end in fetal death, and chronic miscarriage occurs with a frequency of 1 in 300 cases. Hormonal disorders, infectious diseases of the uterus and vagina, which lead to disruption of placentation, genetic pathologies (5–10% of miscarriages), and autoimmune diseases are well-known causes of threatened miscarriage. However, in half of the cases, the cause of spontaneous miscarriage during the first weeks of pregnancy remains unknown. It is believed to be idiopathic miscarriage can be caused by immunological and biochemical factors [1; 2]. The in-depth study of these disorders, the development of effective and affordable diagnostic tests and treatment methods on this basis continues to be one of the most important issues for obstetricians and gynecologists [3].

It is known that progesterone plays an important role in establishing an adequate immune environment in the early stages of pregnancy, and it has a significant impact on immunological processes through immunosuppressive effects [4]. The liver utilizes excess or "spent" progesterone by binding it to albumin and the transport protein transcortin followed by metabolism in hepatocytes and elimination [5, 6].

Increased lipid peroxidation and insufficient activation of compensatory mechanisms, in particular antioxidant components during pregnancy, contribute to changes in the structural and functional properties of cell membranes, including in the placenta, with a decrease in the synthesis of placental hormones, which may be one of the factors threatening abortion [6]. Progesterone reduces the release of free radicals into the extramitochondrial environment during the work of the respiratory chain. Due to its structural characteristics, it is not a true antioxidant, but high levels of this hormone effectively reduce cellular damage by free radicals.

The role of progesterone in regulating the functioning of metalloproteinases (MMP) has also been confirmed: the hormone increases the level of the MMP enzyme. Fetal membranes contain elevated levels of proMMP-1, proMMP-3, and proMMP-9, but not proMMP-2. Placental formation begins with the invasion and migration of trophoblast cells into maternal tissues with the formation of contact with blood vessels. The trophoblast expresses high levels of MMP-9 [7, 8], while *in vitro* inhibition of MMP or deletion of the *MMP-9* gene in mice inhibited migration and destruction of the extracellular matrix by trophoblast cells [9]. The amnion epithelium secretes MMP-2 *in vitro* and is probably responsible for the secretion of MMP-2 in the amniotic fluid in the second trimester of pregnancy [8; 9]. MMP-1 and MMP-2 are also present in the fetal membranes in unchanged amounts before delivery [10]. MMP-9 protein is found in human amniotic epithelial macrophages, smooth muscle chorion and decidual cells after delivery. After obstetric complications (gestosis, preeclampsia), the invasion process is severely impaired – there is no sufficient vascular remodeling, and only single cytotrophoblast cells are embedded in the vessel walls. It was found that the concentration of MMP-2 ($p < 0.001$) and MMP-9 ($p < 0.001$) in the blood was lower in women with preeclampsia [10].

In vitro experiments by Harris L. K. et al. (2010) showed that MMP-12 is secreted by trophoblast cells and is actively involved in the degradation of elastin in the walls of the spiral uterine arteries [8]. The researchers have found a decrease in MMP-12 mRNA expression in placental tissue cells in the first trimester in women with obstetric complications (gestosis, threatened miscarriage and eclampsia). The investigation revealed that the concentration of MMP-12 in the serum of women in the first trimester of pregnancy was high compared to non-pregnant women [8, 9]. At the same time, in the risk group and in complicated pregnancy, the level of MMP-12 decreased, which demonstrated the high biochemical activity of MMP-12 in the initial stages of placentation. A sharp increase in the concentration of MMP-12 in the blood of pregnant women with preeclampsia indicates compensatory hypersecretion of MMP-12, while a gradual decrease in its concentration was observed in normal pregnancy.

In early pregnancy, tissue inhibitor of proteinases (TIMP) levels are high, with a maximum of TIMP-1 at mid-pregnancy in the uterus, decidual membrane and placenta of mice. In the placenta, the expression of TIMP-2 increases 7-fold after the 14th day of pregnancy in mice. The peak expression of TIMP-1 correlates with the most invasive period of embryonic development. Thus, TIMP-1 is not a critical inhibitor of MMP during pregnancy. Since the activity of MMP is controlled by the physiological inhibitor TIMP-1 [7], and the concentration of MMP can strongly depend on the degree of their dilution in the blood, it is important to analyze not only the absolute concentrations of these enzymes, but also the ratio of enzyme/inhibitor concentrations in the blood [8]. Determining the peculiarities of the functioning and regulation of these enzymes in various diseases will allow us to improve the understanding of pathogenesis and to substantiate and implement new methods of treatment in practical medicine.

The aim of the study is to analyze the level of the matrix metalloproteinase type 9 (MMP-9) and tissue inhibitor metalloprotease type 1 (TIMP-1) in the pregravid period and the first trimester of pregnancy in women with the hepatobiliary system (HBS) pathology and a history of miscarriage.

Research materials and methods. The content of serum MMP-9 was determined using a set of reagents "Human MMP-9 ELISA" (Bender MedSystems, Austria) and tissue inhibitor (TIMP-1) – "Human TIMP-1 ELISA" (Bender MedSystems, Austria) by enzyme-linked immunosorbent assay. Serum levels of MMP-9 and TIMP-1 were determined in 88 patients, including 8 women of the control group and 80 women with a burdened obstetric history of chronic HBS diseases (43 pregnant women and 37 women in the pregravid period). All women provided written informed consent to participate in the study. The study adhered to the principles of the World Medical Association's Code of Ethics of the (Declaration of Helsinki).

Determination of the reference norm for metalloproteinase and tissue inhibitor was performed in 8 healthy women from the donors group (Table 1)

Table 1

Indicators of the reference norm of matrix metalloproteinase type 9 (MMP-9) and tissue inhibitor of metalloproteinases type 1 (ng/ml)

Indicator	Norm	Limits of fluctuations
TIMP-1, blood serum	880.5 ± 30.0	820–965
MMP-9, blood serum	116.0 ± 4.5	110–129

Research results and discussion. Metalloproteinases are involved in the development and regulation of the inflammatory response by regulating the migration of macrophages and lymphocytes, vascular permeability, and the activity of inflammatory mediators such as cytokines and chemokines. The role of extracellular proteinases is to activate and regulate tissue remodeling. The value of type 9 MMP in pregnancy is determined by the course of implantation, placentation, and also affects the invasive ability of the trophoblast in the endometrium [8, 9].

The level of serum MMP-9 in patients with impaired liver function during the first trimester of pregnancy was 2.17 times higher than in women with physiological pregnancy (control group (168.0 ± 7.8) ng/ml; $p < 0.001$). In case of threatened miscarriage, the level of proteolysis enzyme increased 1.37-fold compared to women in

Table 2

Levels of matrix metalloproteinase type 9 and its inhibitor of metalloproteinase type 1 in blood serum during gestosis in patients with a burdened premorbid background (M ± m)

Indicator	Control group	Pregnant women with a complicated obstetric anamnesis (n = 39)		p
		study group (n=20)	women with the risk of miscarriage (n = 19)	
MMP-9, ng/ml	168.0 ± 7.8	364.0 ± 21.0*	498.0 ± 16.8*	< 0.001
TIMP-1, ng/ml	832 ± 15	854.0 ± 14.3	723.0 ± 10.4*	< 0.001
MMP-9/TIMP-1	0.20 ± 0.08	0.43 ± 0.10	0.69 ± 0.09*	> 0.05

Note. * – a significant difference between the group of pregnant women with a burdened obstetric history and the control group p < 0.01.

the study group (p < 0.001) and even more compared to the index in physiological pregnancy (almost threefold; p < 0.001) (Table 2).

Thus, in pregnancy complicated by miscarriage, the content of the biochemical factor increases. The obtained results demonstrate an increase in MMP-9 activity in the initial stages of placentation. High secretion of the biochemical factor causes changes in the proteolytic activity of the trophoblast, which leads to violation of the structure and functioning of the uteroplacental complex [10].

The activity of tissue enzymes is regulated by their specific inhibitors – TIMP, which inhibit the activity of MMP. All the patients with a burdened premorbid background in the first trimester of pregnancy had increased level of TIMP-1 in the blood serum, which is a sign of extracellular collagen accumulation and a risk factor for the development of sclerotic changes in trophoblast tissues.

The level of TIMP-1 in the study group had a tendency to increase – (854.0 ± 14.3) ng/ml versus (832 ± 15) ng/ml; p = 0.29). It should be noted that in the group of women with a threat of pregnancy termination, the serum TIMP-1 level decreased 1.17 times compared to the study group (p < 0.001), and was also 1.14 times less than the control group (p < 0.001). Minimal values of TIMP-1 in the blood serum (less than 650 ng/ml) were detected in 8 (42.1%) patients, premature termination of pregnancy was diagnosed within 10–12 days. A decrease in TIMP-1 activity (less than 680 ng/ml) can be considered a prognostic factor for unfavorable pregnancy outcome in the early stages (first trimester). Therefore, an increase in the activity and content of MMP-9 against the background of a decrease in TIMP-1 disturbs the normal process of blastocyst invasion. It is important to analyze not only the absolute amount of MMP and the activity of its inhibitor, but also the ratio of enzyme/inhibitor concentration. The ratio of serum MMP-9/TIMP-1 in the study group doubled relative to the control group (0.43 ± 0.10 versus 0.20 ± 0.08; p = 0.08), in case of a threatened miscarriage – 3.45 times (p < 0.001). As a result, the detected increase in the concentration of serum proteolysis enzymes (MMP-9) and a decrease in their tissue inhibitor (TIMP-1) in patients with a burdened obstetric history in the presence of chronic HBS diseases at the early period of gestation indicate the activation of the processes of extracellular matrix destruction and allow predicting pregnancy complications, in particular miscarriage.

The patients with nonalcoholic steatohepatitis (NASH), during long-term follow up had progression of not only the inflammatory process, but also fibrosis, and this process occurs without clinical manifestations. MMP-9 is associated with chronic inflammatory diseases, tissue remodeling and repair, and cytokine processing. Overexpression of MMP-9 is observed in different pathological conditions characterized by excessive fibrosis (experimental biliary fibrosis, chronic pancreatitis, pulmonary fibrosis) [9].

The results of the study prove that the level of serum MMP-9 in the examined patients with chronic HBS pathology in the pregravid period 2.66 times significantly exceeded the value of the intercellular matrix in healthy individuals (with a normal value (116 ± 4.5) ng/ml, p < 0.001) and averaged (308.0 ± 19.4) ng/ml. The activity of TIMP-1 in the blood serum of patients in the study group increased on average 1.30 times (a normal value (880.5 ± 30.0) ng/ml; p < 0.001), which was equal to (1131.0 ± 37.2) ng/ml. Thus, the value of the ratio of MMP-9/TIMP-1 also increased and was 0.27 ± 0.06, i.e., twice as high as the norm (the norm of 0.13 ± 0.08; p = 0.17) (Fig. 1).

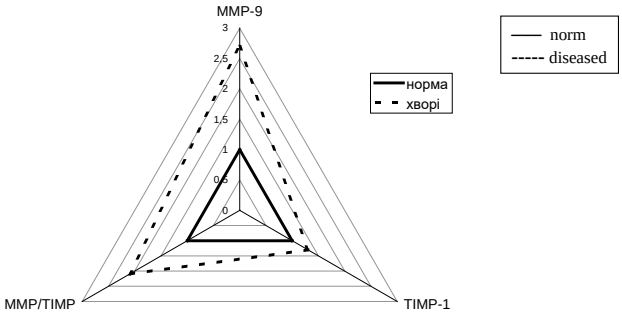

Fig. 1. Level of proteolysis enzyme and its inhibitor (MMP-9 and TIMP-1) in the blood serum of patients with chronic diseases of hepatobiliary system in the pregravid period

As a result, biochemical markers of liver fibrosis progression, such as the increase in tissue proteolysis enzymes and their inhibitors, were found in patients with chronic biliary diseases. Hence, MMP-9/TIMP-1 can be considered as a potential non-invasive marker for the diagnosis of liver fibrosis.

The analysis of biochemical proteolysis factor (MMP-9 and its tissue inhibitor TIMP-1) in the blood serum of patients with chronic biliary diseases was performed depending on the severity of the pathological process in the liver parenchyma (Table 3).

Minimal changes in serum MMP-9 were noted in liver steatosis, its content increased 1.38 times (p < 0.001) averaging (232.0 ± 8.2) ng/ml. The patients with clinical and instrumental signs of NASH had a significant increase in the content of MMP-9 (2.29 times; p < 0.001 compared to

Table 3

Concentration of matrix metalloproteinase type 9 and its inhibitor metalloproteinases type 1 in the blood serum of patients with diseases of the hepatobiliary system depending on the severity of the pathological process in the liver (M ± m)

Indicator	Norm	Examined patients with chronic HBS pathology (n = 39)	
		NASH, exacerbation stage (n = 20)	Liver steatosis (n = 19)
MMP-9, ng/ml	116.0 ± 4.5	384 ± 6.5	232 ± 8.2
TIMP-1, ng/ml	880 ± 30	1320 ± 29.7	941 ± 14.5
MMP-9/TIMP-1	0.13 ± 0.08	0.29 ± 0.05	0.25 ± 0.03

the norm) and was (1320.0 ± 29.7) ng/ml, which is associated with the presence of active inflammatory process in the liver and damage to endothelial permeability.

It should be noted that a correlation between the level of the pro-inflammatory cytokine TNFα and the level of MMP-9 was established ($r = +0.46$; $p < 0.05$). Taking into account that TNFα triggers the synthesis of MMP-9, it can be stated that with increase in its activity, MMP-9 is activated, which may indicate the progression of immunological disorders in the liver tissue and transition of steatosis to steatohepatitis [9, 10].

The activity of the endogenous proteolysis inhibitor TIMP-1 in liver steatosis increased 1.07 times compared to the norm ($p = 0.08$) and amounted to (941.0 ± 14.5) ng/ml. The multiplicity of TIMP-1 increase in the blood serum of patients with NASH was one and a half times higher than normal ($p < 0.001$). Therefore, an increase in TIMP-1 indicates an increase in the degree of fibrosis. It has been proven that TIMP activity depends on the microenvironment of healthy tissues.

The value of the MMP-9/TIMP-1 ratio increased regardless of the severity of the inflammatory and fibrotic process in the liver (see Table 3). The investigated index in the examined patients with hepatic steatosis increased almost twice (at the norm 0.13 ± 0.08; $p = 0.17$), and in NASH – 2.23 times ($p = 0.10$), which was equal to 0.25 ± 0.03 and 0.29 ± 0.05, respectively.

Thus, the progression of the fibrosis process in the liver is associated with an imbalance in the MMP-TIMP system, which can lead to disruption of the structure of the extracellular matrix of the organ parenchyma.

Conclusions

1. Impaired metalloproteinase activity during pregnancy in women with miscarriage and chronic diseases of the hepatobiliary system in the history indicate their influence at the beginning of the placentation process.

2. Activation of MMP-9 indicates the progression of immunological disorders in liver tissues and the transition of steatosis to steatohepatitis.

3. Imbalance in the MMR-TIMR system is associated with the progression of the fibrosis in the liver, which can lead to disruption of the structure of the extracellular matrix the organ parenchyma.

Conflict interests

The authors declare no conflict of interest.

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