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ЖУРНАЛ КАРДИОРЕСПИРАТОРНЫХ ИССЛЕДОВАНИЙ

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РОЛЬ АНГИОГЕННЫХ ФАКТОРОВ РОСТА В ПАТОГЕНЕЗЕ ПРЕЖДЕВРЕМЕННЫХ РОДОВ У БЕРЕМЕННЫХ НА ФОНЕ НЕДИФФЕРЕНЦИРОВАННОЙ ДИСПЛАЗИИ СОЕДИНИТЕЛЬНОЙ ТКАНИ С ПРОЛАПСОМ МИТРАЛЬНОГО КЛАПАНА

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АННОТАЦИЯ

Провести анализ изменений соотношения проангиогенных и антиангиогенных факторов у женщин с признаками преждевременных родов на фоне недифференцированной дисплазии соединительной ткани (НДСТ). Обследованы 66 пациенток с 30-34 недельным сроком беременности, у которых имелись признаки НДСТ. Определение растворимых форм VEGF, VEGF-R1, VEGF-R2, MMP-2, MMP-9, TIMP-1 и TIMP-2 в сыворотке крови осуществляли с помощью иммуноферментного анализа с использованием стандартных тест-систем фирмы Bender MedSystems GmbH (Австрия) и R&D Systems (США). По полученным результатам рассчитывали соотношения про- и антиангиогенных факторов. Полученные результаты свидетельствуют о возможности использования анализа лиганд/рецепторных пар в характеристике процессов ангиогенеза для прогнозирования преждевременных родов. Причем наиболее информативными являются соотношения VEGF/VEGF-R1, VEGF/VEGF-R2 и MMP-9/TIMP-1. Характер изменения соотношений VEGF/VEGF-R1 и VEGF/VEGF-R2 внутри групп в исследуемые сроки беременности предполагает наличие единого механизма, регулирующего взаимоотношения VEGF и его рецепторов VEGF-R1 и VEGF-R2. Соотношения же PLGF/VEGF-R1 и MMP-2/TIMP-2 являются малоинформативными. Выявленное в настоящем исследовании различие в соотношении факторов, регулирующих ангиогенез гестационного периода между группами с удачными и неудачными перинатальными исходами может свидетельствовать об особенностях, а в случае группы с преждевременного излития околоплодных вод – о нарушениях ангиогенеза сосудистой системы плаценты и эмбриона на фоне НДСТ.

Ключевые слова: ангиогенез, беременность, соотношения про- и антиангиогенных факторов, VEGF/VEGF-R1, VEGF/VEGF-R2, MMP-9/TIMP-1.

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THE ROLE OF ANGIOGENIC GROWTH FACTORS IN THE PATHOGENESIS OF PRETERM LABOR IN PREGNANT WOMEN ON THE BACKGROUND OF UNDIFFERENTIATED CONNECTIVE TISSUE DYSPLASIA WITH MITRAL VALVE PROLAPSE

ANNOTATION

To analyze changes in the ratio of pro-angiogenic and anti-angiogenic factors in women with signs of premature birth against the background of undifferentiated connective tissue dysplasia (UCTD). Sixty-six patients with 30-34 weeks of pregnancy were examined with signs of UCTD.

The soluble forms of VEGF, sVEGF-R1 (sFlt-1), sVEGF-R2 (sKDR), MMP-2, MMP-9, TIMP-1 and TIMP-2 were determined in their sera by enzyme immunoassay, by using the standard test systems (Bender MedSystems GmbH (Austria) and R&D Systems (USA)). The findings were used to calculate the ratios of pro- and antiangiogenic factors. The findings suggest that analysis of ligand/receptor pairs may be used to characterize the processes of angiogenesis in premature birth. Moreover, the VEGF/VEGF-R1, VEGF/VEGF-R2, and MMP-9/TIMP-1 ratios are of the greatest informative value. The nature of intragroup changes in the VEGF/VEGF-R1 and VEGF/VEGF-R2 ratios in the examined pregnancy periods proposes that there is a unified mechanism that regulates relationships between VEGF and its receptors VEGF-R1 and VEGF-R2. The PLGF/VEGF-R1 and MMP-2/TIMP-2 ratios are of low informative value. The difference found in this study in the ratio of factors regulating gestational angiogenesis between groups with successful and unsuccessful perinatal outcomes may indicate features, and in the case of a group with premature excretion of amniotic waters, disorders of the angiogenesis of the vascular system of the placenta and embryo against the background of UCTD.

Keywords: angiogenesis, premature birth, ratio of pro- and antiangiogenic factors, VEGF/VEGF-R1, VEGF/VEGF-R2, MMP-9/TIMP-1, undifferentiated connective tissue dysplasia.

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MITRAL KLAPPAN PROLAPSI BILAN DIFFERENTIALANMAGAN BIRIKTIRUVCHI TO'QIMA DISPLAZIYASI FONIDA HOMILADOR AYOLLARDA MUDDATIDAN OLDIN TUG'ILISH PATOGENEZIDA ANGIOGEN O'SISH OMILLARINING ROLI

ANNOTATSIYA

Differentsialanmagan biriktiruvchi to'qima displaziyasi (DBTD) fonida erta tug'ilish belgilari bo'lgan ayollarda pro-angiogen va antiangiogen omillar nisbatidagi o'zgarishlarni tahlil qilish. DBTD belgilari bo'lgan 30-34 haftalik homiladorlik davridagi 66 nafar bemor tekshirildi. Qon zardobida VEGF, VEGF-R1, VEGF-R2, MMP-2, MMP-9, TIMP-1 va TIMP-2 ning eruvchan shakllarini aniqlash Bender MedSystems GmbH (Avstriya) standart sinov tizimlaridan foydalangan holda ferment immunoassay yordamida amalga oshirildi. Olingan natijalar asosida pro- va antiangiogenik omillarning nisbati hisoblab chiqilgan. Olingan natijalar erta tug'ilishni bashorat qilish uchun angiogenez jarayonlarini tavsiflashda ligand / retseptor juftlarini tahlil qilishdan foydalanish imkoniyatini ko'rsatadi. Bundan tashqari, eng ma'lumotli VEGF/VEGF-R1, VEGF/VEGF-R2 va MMP-9/TIMP-1 nisbatlaridir. Homiladorlikning o'rganilayotgan davrlarida guruhlarda VEGF/VEGF-R1 va VEGF/VEGF-R2 nisbatlarining o'zgarishi tabiiy VEGF va uning VEGF-R1 va VEGF- o'rtasidagi munosabatlarni tartibga soluvchi yagona mexanizm mavjudligini ko'rsatadi. R2 retseptorlari PLGF/VEGF-R1 va MMP-2/TIMP-2 nisbatlari ma'lumotga ega emas. Ushbu tadqiqotda aniqlangan muvaffaqiyatli va muvaffaqiyatsiz perinatal natijalarga ega bo'lgan guruhlar o'rtasidagi homiladorlik davrining angiogenezini tartibga soluvchi omillar nisbatidagi farq xususiyatlarni va amniotik suyuqlikning erta yorilishi bo'lgan guruhda, angiogenezning buzilishi DBTD fonida platsenta va embrionning qon tomir tizimini ko'rsatishi mumkin,

Kalit so'zlar: angiogenez, homiladorlik, pro- va antiangiogen omillar nisbati, VEGF/VEGF-R1, VEGF/VEGF-R2, MMP-9/TIMP-1.

Relevance. Undifferentiated connective tissue dysplasia (UCTD) is a genetically heterogeneous group of abnormalities that can serve as the basis for the formation of various chronic diseases. At present, endothelial dysfunction and collagen formation disorder play an important role in the formation of UCTD. According to the authors, all this is the reason for the progression of factors leading to premature births in women with UCTD. It should be noted that endothelial dysfunction is the cause of circulation disorder in the body. This is due to the production of vasoactive and thrombogenic compounds that provoke vascular spasm or vascular thrombosis. The main factor damaging vascular endothelium is: increased levels of cytokines (IL-1 β , TNF- α , IL-8), which leads to the development of hypoxia.

Developing hypoxia induces increased synthesis of the glycoprotein vascular-endothelial growth factor (VEGF), regulating neo-angiogenesis and, as a consequence, increasing of blood vessels. Lowering VEGF levels under hypoxia conditions causes endothelial apoptosis leading to lumen obstruction and vascular regression as they are key factors regulating gestational period angiogenesis. Collectively, this may be an important trigger mechanism for the activation of destructive metalloproteinase's (MMPs) and their tissue inhibitors (TIMPs). The specific effect on endothelium is achieved by interaction with VEGF-R1 receptors, VEGF-R2, VEGF-R3 expressed on endothelial cells. By specifically hydrolyzing extracellular matrix components and basal membrane collagen, MMPs promote cell invasion and migration, thereby facilitating the formation and development of the vascular network. The activation of the latest brings to the degradation of the extracellular matrix, which causes the

progression of the severity of the UCTD, which in turn will lead to premature exfoliation of the amniotic waters (PEAW).

Existing models that describe the interaction of VEGF family members with receptors, as well as MMP family members with inhibitors, do not provide quantitative binding characteristics in the receptor/ligand system for these molecules. Therefore, it is not possible to use any indices or ratios characterizing the dynamic situation described in the selected time interval as a pro-angiogenesis state. To describe such situations, it is possible to introduce "surrogate" indices characterizing the value of the ligand/receptor ratio as being most adequate for characterizing any process, particularly angiogenesis. The use of such ratios can be used to calculate the risk of complications of pregnancy, for example PEAW.

The purpose of this study was to analyze changes in the ratio of pro-angiogenesis and anti-angiogenesis factors in women with signs of premature birth against the background of undifferentiated connective tissue dysplasia (UCTD).

Subjects and methods. The prospective study included 66 patients with 30-34 weeks of pregnancy with signs of UCTD. The control group was 20 patients with physiological course of pregnancy. The main group included 46 pregnant women with signs of UCTD (myopia, flatness, scoliosis, joint hypermobility, mitral valve prolapse of the I degree and abnormally located chord). All these women had PEAW in history and threat of termination of current pregnancy. Of these, 16 patients had premature delivery in the form of premature placenta detachment (main subgroup A) and 30 patients had PEAW (main subgroup B).

In patients of main subgroup A, B and control group,

angiogenesis factors in peripheral blood samples were examined within 7-8 weeks, 11-14 weeks and 30 weeks of pregnancy. The criteria for inclusion were two or earlier pregnancy losses in history, PEAW, a single-fetal pregnancy that occurred in the natural cycle. The diagnosis of the pregnancy period was carried out on the basis of the ultrasonography study and the level of the β subunit of chorionic gonadotropin. Definition of the soluble forms VEGF, sVEGF-R1, sVEGF-R2, MMP-2, MMP-9, TIMP-1, TIMP-2 in serum of blood was carried out by the linked immune-sorbent assay (ELISA) with using of standard test systems of Bender MedSystems GmbH (Austria) and R&D Systems (USA). The validity of the differences in the average values of the calculated values was evaluated by means of a two-sample *t*-test with different dispersions. The differences were considered valid at a value level of $p < 0.05$.

Results of research. VEGF relation to a receptor of sVEGF-R1 (VEGF/VEGF-R1) at patients of control group is characterized on pregnancy term up to 36 weeks. A value equal to 0.562, which is significantly higher than the value of this ratio in A and B subgroups of the main group, which is 0.178 and 0.0312, respectively. It is interesting to note that dynamics of change of a ratio of VEGF/VEGF-R1 in the main subgroup A has similar trends with control group. In basic subgroup B, the ligand/receptor pair values were significantly reduced except for the last survey period (0.0086) compared to control (0.006) and subgroup A (0.0024).

VEGF relation to a receptor of sVEGF-R2 (VEGF/VEGF-R2) has similar dynamics. Thus, in the period up to 7-8 weeks of pregnancy there are significant differences only in subgroup B (0.0023) compared to the control group (0.020) and under group A (0.011). At that by dynamics of change of ratio the control and main A groups coincide, at the same time finding reliable differences at the interval of 11-14 weeks. Subgroup B is characterized by minimal values of the ratio, which is significantly different in terms up to 11-14 and in 30-32 weeks of pregnancy compared to other groups, without having reliable differences within the group at all studied terms of pregnancy. It is known that the effect of VEGF on endothelial cells is accomplished by binding to VEGF-R1 and VEGF-R2 receptors.

Activation of VEGF-R2 leads to stimulation of angiogenesis by triggering proliferation, migration and differentiation, as well as inhibition of endothelial cell apoptosis. The action of activated VEGF-R1 receptor is realized in activation of intercellular interactions, branching of vascular network and processes of trophoblast invasion into spiral arteries. According to literature, the key receptor for VEGF in pregnancy is VEGF-R2. In our research the received

values / receptor couples for VEGF and receptors of sVEGF-R1 sVEGF-R2 allow to assume a ligand that the nature of change of a ratio of VEGF/VEGF-R1 and VEGF/VEGF-R2 in all points of observation reflects realization of the processes which are carried out by these factors, and being significant for the normal course and the result of pregnancy. As formation of active VEGF/VEGF-R1 complex influences, mainly, processes of a differentiation and migration of cages of the trophoblast and also regulates a trophoblast invasion, the maximum values observed in control group in all points of observation, reflect the optimum balance of factors which is established in a dynamic system in the studied terms.

It is revealed that at UCTD reduced values of a ratio VEGF/VEGF-R1 in the main subgroup A (by 3 times) and especially in subgroup of B (more than by 18 times), statistically significantly reflect the insufficient activation answer and reduced functional activity of cages that is critical for pregnancy. The nature of change of a ratio in VEGF/VEGF-R2 system allows to assume existence of similar VEGF/VEGF-R1 of regularity of functioning of the given a ligand / receptor couple in circulation. In the development of preeclampsia, the sVEGF-R2 in peripheral blood decreases, while the sVEGF-R1 increases. Apparently, the maximum value of a ratio VEGF/VEGF-R2 in control and the main A groups demonstrates high activity of factors in processes of a vascularization of placenta and embryonic angiogenesis. A reliably reduced ratio in the main subgroup B is likely to indicate disorders in the structure of the amniotic shell and placenta, also inadequate angiogenesis during gestation.

The nature of change of a ratio of factors of VEGF/VEGF-R2 in the studied terms in all groups testifies to exclusive importance of the given a ligand / receptor couple on all weeks of a gestation. Analysis of the results showed significant differences for the 30-32 week MMP-9/TIMP-1 ratio for major subgroup B (0.91) compared to the control group (1.48). A significantly reduced value of this ratio in this group may indicate the characteristics of degradation and autolysis processes in UCTD.

Critical decrease in a ratio of VEGF/VEGF-R1 and VEGF/VEGF-R2 is significant on term up to 14 weeks of pregnancy. At the same time, a decrease in the MMP-9/TIMP-1 ratio of 30 - 32 weeks may be one of the factors that signal the development of critical effects leading to PR.

Conclusions. Thus, the results show the possibility of using ligand/receptor pair analysis in characterizing pathological processes in pregnancy. This study found differences in the ratio of factors regulating gestational period angiogenesis between groups with successful and unsuccessful perinatal outcomes, especially in patients with signs of UCTD, which may have prognostic significance.

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